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VOLUME I
1912-1920

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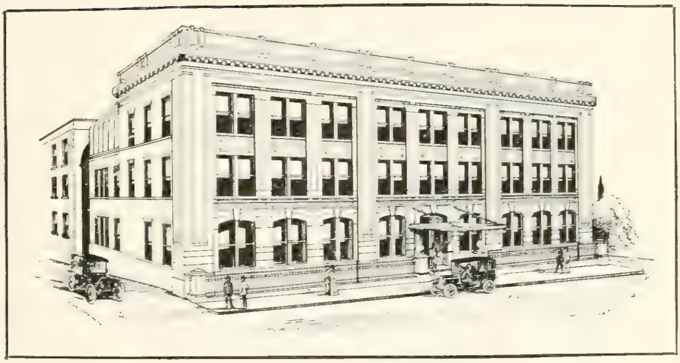

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# PHYSIOLOGICAL DRUG TESTING AND THE UNITED STATES PHARMACOPŒIA

C. C. HASKELL, A. B., M. D.

[Read before the section on Scientific Papers of the American Pharmaceutical Association at the meeting held in Boston, Mass., Aug. 14-18, 1911.]

The chemical assay of aconite, cannabis indica, ergot, and the "heart tonics" is not practicable. In view of the great importance of some of these drugs, the attempt has been made to standardize them by tests upon the lower animals. That methods of physiological drug testing are of undoubted value is generally admitted, but that by these methods sufficient accuracy is secured to warrant their adoption as official methods of assay is not so universally believed. It has seemed to me that a discussion of physiological assaying as related to the Pharmacopœia is particularly appropriate at this time.

Roughly, the methods of physiological testing of drugs may be divided into two classes. In the first of these, a determination is made of the amount of drug necessary to cause the death of an animal; in other words, we endeavor to ascertain the killing power of the particular drug. In the second class, the attempt is made to measure the effect of the drug upon the function of living tissue.

It would seem, on theoretical grounds, that the methods based upon the determination of the lethal dose are less likely to give reliable results than are those methods where the attempt is made to measure some peculiar physiological action of a drug; some action which has been shown clinically to be of therapeutic value. If, however, it is proven that the lethal action is always due to the therapeutically active principles and that the action is always upon the same vital centres, then this theoretical objection is removed. Until then, however, as has already been pointed out, simply because one specimen of drug is twice as poisonous as a second gives us no reason for assuming that the former is twice as active therapeutically as the latter.

The prime requisite for any method of assay is, of course, accuracy. The degree of accuracy being the same, methods showing cheapness, simplicity and rapidity are to be preferred, and it will be of value for us to keep these points constantly in mind.

For the assay of aconite two methods have been proposed. The first of these belongs to class two of our arbitrary division, and depends upon the fact that aconite has the property of so stimulating sensory nerve terminals as to cause a tingling sensation. Dr. Squibb endeavored to learn at how great dilution an aconite preparation was still capable of causing this sensory reaction when the solution was held in the mouth. The other method is simply a lethal dose method; guinea pigs, frogs, or other animals being used in the experiments.

Dr. Squibb's method is unsatisfactory because the personal equation is apt to influence the results. A very weak solution and a strong imagination will enable one observer to experience the same tingling that a second would experience with a stronger solution and a less active imagination. The lethal dose method has also not been carefully enough studied to prove its reliability, so an intelligent discussion is not practicable.

So far as I am aware, the only assay method for cannabis indica is that proposed by Dr. Houghton or some modification of Houghton's method. In sufficiently large dose, cannabis indica is capable of causing intoxication in a dog, first evidenced by a swaying from side to side when the animal stands. By determining the amount of the drug necessary to cause the first evidences of intoxication, it is claimed that fairly accurate results can be secured.

The personal equation of the observer is again a factor to be reckoned with. What constitutes the first symptoms of intoxication? One man may select the slightest swaying, in which event a normal dog may often deceive him; another observer may demand very marked evidences, for which a much larger dose is required. Moreover, the reaction of the dogs is apt to vary from the fact that minimal exciting doses are given, these small doses being more apt to bring out individual peculiarities than are large ones. It is also true that accurate standardization of cannabis indica is not so essential as it is in the case of some other drugs, owing to the low toxicity of canabis and the number of efficient substitutes that we have.

In ergot we have a drug of great value and one of such complex character that the chemical assay will probably never be satisfactory. Keller's method was formerly believed accurate, but the consensus of opinion seems that the results secured by the determination of the "cornutin" are not to be relied upon. The method proposed recently by Dr. Wood has also been found wanting, and we are forced to turn to physiological tests.

All of the methods now commonly used for the physiological assay of ergot are based upon attempts to measure directly the therapeutic action of the drug as shown by its effect upon the function of the lower animals or upon their excised organs. The cock's comb method, which owes its prominence to Dr. Houghton, depends upon the power of most fresh ergot preparations to cause, when administered to a chicken, changes in the comb which may be so pronounced as to result in gangrene. It was formerly believed that this effect upon the comb was due to the vaso-constrictor action of ergot, but this view has recently been questioned. If, as Ellinger claims, identical changes can be produced by cantharidin, it is evident that the action cannot be considered characteristic of ergot, and the method can be thought reliable only when it has been shown by careful clinical tests that this gangrene producing power of ergot as measured on the chicken runs parallel with the therapeutic activity of the drug. The work of Edmunds and Hale, showing the agreement between the cock's comb test and the test upon the uterus of one of the lower animals is very suggestive.

The cock's comb test has been adversely criticized, but it would seem that in some instances the poor results were due not so much to weakness of the method as to faulty technic. In our laboratory we aim to use fowls of the same breed and of as near the same age and weight as is practicable. The drug, in the form of a fluid extract, is injected into the pectoral muscles or into one of the wing veins. If the fowls are picked up at random and not kept under precisely similar conditions, accuracy cannot be hoped for. It has seemed to us, also, that the oral administration of ergot to chickens is objectionable, because certain of the active constituents are not supposed to be absorbed from the gastro-intestinal canal.

It is certainly true that the reaction not being automatically recorded is a serious disadvantage. Here, as in some of the other tests I have mentioned, the individual bias of the observer is apt to interfere with accuracy, and the result secured by different men may differ widely owing to the different degrees of reaction aimed at.

Some years ago, Dixon proposed that the vaso-constrictor action of ergot, as evidenced by the rise in the blood pressure of a mammal, be made use of in the attempt to standardize ergot preparations. This method has been extensively used in England, but seems to have at least two serious disadvantages. In the first place, it has been shown (Goodall, Dale, Edmunds and Hale) that the effect upon the blood pressure and upon the uterus *are not always parallel.* So far as I can learn, there is no experimental evidence for supposing that the two sets of effects (upon the blood pressure and upon the uterus) are parts of a wide-spread general action of the drug, and the assumption that vaso-constrictor action is an index of the potency of uterine action is not justified. Dale and Laidlaw have pointed out that B-iminazolylethylamine, one of the constituents of ergot, causes tetanic uterine contraction with a coincident fall in blood pressure.

Even were this method to represent a measure of the therapeutic value of ergot, it does not seem practicable to secure an accurate measure by means of it. Unlike adrenalin, ergot, in an amount large enough to produce an appreciable rise of blood pressure, has a very lasting effect and it is not practicable to make more than one injection into the same animal for purpose of comparison. If the same preparation be given to a number of different animals, it is apparent that these different animals will show great differences in the blood pressure effects.

Finally, there are the two methods in which the effect of ergot upon the uterus itself is observed. In Kehrer's procedure, the organ is excised and the ergot in solution brought in contact with it. In the method advocated by Dr. Edmunds, the movements of the uterus of a cat are observed by means of opening the abdomen of an anæsthetized animal in salt solution, the drug being injected intravenously.

There are several points which seem to render these methods undesirable. In the first place, both methods are technically rather difficult. Again, it is claimed that the condition of the uterus as regards parturition influences the reaction to ergot. Certain of the active constituents isolated by Barger stimulate the parturient; inhibit the virgin uterus. Then spontaneous movements often set up and cease from some unknown cause.

The most important drug on our list is digitalis. The fact that digitalis is so widely used and that variations in strength on either side of a mean is liable to have disastrous consequences renders it extremely desirable to secure some means of standardizing the medicinal preparations of this drug. A discussion of digitalis would also include apocynum, convallaria, strophanthus, and squill, since there is at present no satisfactory chemical assay for any of these.

The lethal dose method for testing digitalis was the first method to be employed commercially in attempting to standardize drugs by pharmacological experiments. To Dr. Houghton belongs the credit for this important step, which he took when he devised his 12-hour frog method for the assay of the heart tonics. This method is based upon the determination of the lethal dose of digitalis for frogs.

Dr. Houghton had also used guinea pigs in standardizing the heart tonics, but found them unsatisfactory. Dr. Reed, however, believed more accurate results could be secured by using guinea pigs rather than frogs.

Dr. Hatcher recently announced his cat method, by which he claimed great accuracy could be obtained,while, at the same time he considers the method to be simple and cheap.

I have already mentioned briefly a possible source of error is testing a drug by lethal dose methods. Does the therapeutic value of digitalis run parallel with its toxicity for lower animals? Is, as has been claimed, this lethal action of digitalis upon a guinea pig or a cat simply an exageration of the therapeutic action of the drug, the poisoning of the heart being the cause of death? Dr. Reed, Dr. Githens, Dr. Hatcher, all claim this to be the case. Cushny, on the other hand, states that even the glucosides of therapeutic value act largely upon the central nervous system; while Edmunds and Hale believe that the death of mammals after digitalis poisoning sometimes results from failure of the heart; sometimes from failure of the respiration. Nestor has come to the conclusion that death of rabbits from the lethal action of the glucosides is always due to respiratory failure, and, in some instances, he was able to save animals from the "dose always fatal" by the maintainence of artificial respiration. From a few experiments upon guinea pigs, I have always found the heart beating strongly after complete cessation of respiration and apparent death of the animal. In a series of experiments carried out in our laboratory by Mr. Eckler using Dr. Hatcher's method, the respiratory movements of the cats were continued after apparent cessation of the heart beat, but the respiration was often seriously embarrassed before any appearances of cardiac failure. When "pure principles" are used, it is probable that the death of frogs results from cardiac poisoning, and, consequently, this method would represent a measure of the therapeutic action of such pure principles.

If we could assume that it was the bodies of therapeutic value alone that caused the death of the animal, it would not be so important how death was caused, provided there was shown to be a constancy in the dosage required. But in the Galenical preparations of digitalis we have very complex mixtures. Suppose, as Dr. Hale pointed out, there should be a relative excess of digitonin present in a tincture of digitalis. Owing to the lethal action of this glucoside, very misleading results would be obtained by using a lethal dose method, and a preparation not only therapeutically weak, but capable of causing serious harm if used clinically might be considered of good strength.

On theoretical grounds, Dr. Cushny's frog heart method seems to avoid these objectionable features. This is a qualitative test of undoubted value, for I do not know of any substances present in digitalis leaves capable of producing the typical "digitalis heart" except the glucosides of the heart tonic series which are of value therapeutically. Even could we measure accurately the toxicity of digitalis for lower animals, it cannot be claimed that we are always sure of measuring the therapeutic activity of the drug, for not only may a relative excess of the undesirable digitonin be present, but it is possible that injuriously acting decomposition products may arise with the aging or manipulation of a preparation, and it is certainly conceivable that as a tincture ages and deteriorates , it may increase in toxicity for mammals and at the same time not only lose in therapeutic efficiency but actually acquire an increasing power to do harm if used clinically. By observing the action on the frog's heart, we gain positive information concerning the desired glucosides, for they alone, so far as I can learn, are capable of bringing about the characteristic changes in the heart. Focke's method also possesses this advantage, but the barbarity it necessitates will prevent its adoption. The perfusion of the isolated heart involves a complicated technic and cannot be considered very accurate.

It has been urged that frogs vary markedly in their reaction to digitalis, but from my limited experience, I can agree with Hale and Focke that the unsatisfactory results secured are rather due to lack care on the part of the operator than to unfitness of frogs. When we see the concordant results of Famulener and Lyons and Hale we cannot but feel that the method of Cushny is accurate. It may be of interest to state that with ouabain, a pure substance, almost the same results were secured in Dr. Houghton's laboratory and ours, these results being obtained in each place without knowledge of the work in the other laboratory. To eliminate the possibility of variations due to season or locality, a definite chemical compound, such as strophanthin, suggested by Dr. Houghton, or, preferably, ouabain may be used, and the frogs themselves "standardized."

On the other hand, some authorities would have us think that mammals do not show appreciable individual variation. In the hands of Dr. Hatcher, remarkably uniform results were secured in the early work with his cat method, but he has recently reported an error of more than 50 per cent.

I have been unable to find the report of any very satisfactory evidence showing that guinea pigs react uniformly to digitalis regardless of age, weight, sex, season, and diet. It would seem advisable to publish such evidence in view of the marked variation sometimes shown by guinea pigs in their resistance to bacterial poisons and the interesting experiments by Dr. Hunt, showing the effect of different diets on the resistance of guinea pigs to poisoning by acetonitrile.

Dr. Houghton has, I believe, found his method involves an error less than 10 per cent. Using "pure principles" Hale has found the one hour method fully as accurate as this. In our own laboratory we have made our work, carried out independently, check within 10 per cent.

There can be no question as to the economy of the different methods. Frogs for an assay cost us about 50 cents. Guinea pigs would cost us about $4.00. Cats could not be secured in Indianapolis in sufficient numbers for our use.

As regards simplicity, there is little to choose between Houghton's and Cushny's method. The guinea pig cannot be handled by one man, while Hatcher's method is quite complicated.

The one hour frog method enables us to complete an assay in, at most, three hours. Houghton's method requires at least twelve hours, as does the guinea pig method. The actual time needed to run one cat, according to Hatcher's method is ninety minutes. If, as seems necessary, three animals are used, the whole day is taken up, the preparation of the animals requiring some time.

Accuracy, cheapness, simplicity, speed. It would seem that in none of these points is Cushny's method excelled. Houghton's method is more time consuming, and it is conceivable that it may give erroneous results when other poisons besides the active glucosides are present in large amount.

It seems that the frog heart method is the only one that has been controlled clinically. Pratt in this country has shown how the therapeutic efficiency of digitalis leaves varied as did their strength as determined by this method. Focke also mentions similar comparisons. The worth of digipuratum, which is standardized by a modification of Cushny's method, has been shown by many clinical tests.

In conclusion, it may be said that in the one hour frog heart method is offered a means of standardizing digitalis which compares favorably with chemical assay methods when the test is carried out with due precautions by trained men.

It would probably be unwise to adopt as official any of the methods now used for the pharmacological assay of aconite, cannabis indica, ergot. Further study is needed before it can be determined which are most suitable, but in the meantime it is very desirable that manufacturers use these methods, thereby insuring more nearly uniform preparations and also acquiring valuable data upon the methods used. *—Department of Experimental Medicine.*

# SUSCEPTIBILITY OF THE GUINEA PIG TO POISONING BY DIGITALIS

A. L. WALTERS, B. S., and C. C. HASKELL, A. B., M. D.

[Read before the section on Scientific Papers of the American Pharmaceutical Association at the meeting held in Boston, Mass., Aug. 14-18, 1911.]

A method of drug assay to be suitable for an official assay process should be accurate. This point outweighs all others, and many objectionable features can be overlooked if the method in question enables us to measure accurately the therapeutic properties of the drug examined. Nevertheless, should two methods show no appreciable difference regarding the degree of accuracy attainable, the cheapness, simplicity, and rapidity of performance come to have considerable influence in the choice between them.

The advocates of the frog methods believe that careful investigation, extending over many years in different laboratories has shown that these methods carried out upon "standardized" animals enable the pharmacologist to estimate the strength of digitalis preparations with an error not greater than 10 per cent. So far as we have been able to learn, the valid objections to the use of frogs are removed when a reliable standard, such as ouabain or Houghton's crystalline strophanthin, is used. Compared with the guinea pig method, proposed by Dr. Reed, the frog methods are much cheaper. In the assay of a digitalis preparation by the one hour frog heart method, we practically never use more than 18 frogs, the cost of which varies from forty to seventy-five cents. Our ignorance of the routine use of the guinea pig method prevents us from stating definitely the cost of an assay. It scarcely seems probable, however, that, on an average, less than six or eight animals would suffice. The guinea pigs we have been able to obtain have cost us from 40 to 75 cents apiece, making the probable cost of an assay from two to six dollars, about eight hundred per cent, greater than the frog method.

Closely connected with the cost, are the rapidity and simplicity of execution. By the one hour frog method, an assay can be completed by one man in three hours at the utmost. By the guinea pig method, at least twelve hours must be consumed, and probably twenty-four. Moreover, an assistant is required in injecting the solution into the guinea pig and accuracy of dosage is more difficult to obtain. It would seem, from the investigation that we have carried out, that an alcoholic strength of 25 per cent. has no appreciable influence on the reaction of frogs to ouabain, so that removal of the alcohol from official digitalis preparations is not necessary for this assay upon frogs, since the necessary dilution accomplishes the desired reduction.

But if it be granted that the guinea pig method is deficient in these points, is the accuracy attainable by it sufficient to counterbalance the less important considerations? Dr. Reed and Dr. Githens are convinced that this is so, and the opinions of such careful investigators are worthy of much consideration.

The question of the lethal action of digitalis upon mammals is of some interest. Dr. Githens believes that guinea pigs die as a result of the action of the drug upon the heart, all the phenomena observed being due to circulatory embarrassment. It would seem advisable to adduce experimental evidence in support of a view which is denied by such men as Edmunds, Hale, Cushny, and, apparently, has been disproven for rabbits by Nestor.

This point, however, is relatively unimportant if it can be proven that the lethal power of digitalis for guinea pigs runs parallel with the therapeutic efficiency of the drug. That such parallelism exists, however, is a dangerous assumption if our knowledge concerning the chemistry of digitalis is correct. It is held that the glucosides tend to break up under certain poorly understood conditions, giving rise, probably, to numerous decomposition products, among which toxiresin and digitalresin are prominent. According to Sollmann, these bodies appear to be more poisonous than the original glucosides, so it is conceivable that an old, detoriorated preparation, one that is not only therapeutically weak but capable of doing serious damage if used clinically would seem of good strength when tested by a lethal dose method. If we are right in the assumption that the therapeutically active glucosides are the only substances present in digitalis preparations which are capable of bringing about the cardiac changes upon which the one hour frog heart method is based, it is evident that this method, being a qualitative as well as quantitative test of digitalis, possesses a great advantage over the lethal dose methods.

Aside from these theoretical considerations what evidence is there for or against the guinea pig as a test animal? So far as we can discover, there have been no reports of experiments carried out to show that the susceptibility of guinea pigs to digitalis poisoning is constant regardless of age, weight, sex, diet, season, or locality. On the other hand, Dr. Houghton has found them less useful than frogs, and, as the result of his earlier work, he discarded guinea gigs for the latter animal in the assay of digitalis.

While the variations in the susceptibility of guinea pigs to other poisons cannot be accepted as positive proof that similar variations will occur when digitalis is the toxic agent, yet such evidence is very suggestive.

Dr. Arms has called attention to the fact that very striking individual variations are encountered when tests are made upon guinea pigs with the virus of rabies or with material containing infectious organisms. Hunt has shown that guinea pigs vary in their susceptibility to acetonitrile according to season and diet. In January, the m. l. d. of acetonitrile per gram guinea pig was 0.20; in July, 0.45; a difference of 125 per cent. One series of pigs fed on green food survived a dose of 0.53 gm., while a second series kept on an oats diet under similar conditions succumbed to a m. l. d. of 0.23; a difference of 130 per cent.

Sudmersen and Glenny found that guinea pigs varied in their susceptibility to diptheria toxin according to season and inversely as to age. In January, the approximate m. l. d. was 0.006; in July, 0.009, a variation of 50 per cent. It is worth noting that these investigators found that the animals resisting power to diptheria toxin was greater in summer and fall, agreeing with Hunt's results when acetonitrile was used.

If similar variations in the susceptibility of guinea pigs to digitalis poisoning should be found present, it is obvious that these animals would be suitable for the assay of the members of the digitalis group only when a standard preparation is used on a control series of pigs, and the method would possess absolutely no advantage over the frog methods, while being much more cumbersome. In view of these facts, we have taken up the study of the susceptibility of guinea pigs to poisoning by members of the digitalis series in the attempt to learn whether this susceptibility is the same in a series of animals kept under similar conditions, is uninfluenced by artificial respiration, by the weight of the animals, by the diet of the animals, or by the season of the year. Further, we wish to investigate the action of old preparations of digitalis upon frogs and upon guinea pigs to see whether the rate of deterioration is the same as determined by the two methods.

In this paper we present only a preliminary report. Two factors, the lack of time and the great difficulty of securing guinea pigs have forced us to postpone much of that we had contemplated doing, but we have secured some rather incomplete data upon a few of the points mentioned.

We found it impossible to obtain a sufficient number of pigs, although we tried dealers in Indianapolis, Terre Haute, Lexington, Chicago and Boston. The pigs we secured were, except when stated otherwise, fed liberally on oats, hay and cabbage, and had access to water constantly.

In these earlier experiments, we have used the crystalline gratus strophanthin or ouabain of Merck. A stock solution of 1 to 1000 was made up with 70 per cent. alcohol, and this was diluted 1:10 with normal salt solution before injection. We realize that there are points of difference in the action of the various members of the digitalis group, but we do not believe that these differences are great enough to vitiate the results we have secured. The solution was injected under the skin of the abdomen by means of the Hitchens syringe by which accuracy of dosage was secured.

Dr. Reed at first considered that any animal receiving a lethal dose would succumb within three hours. The time was subsequently extended to 12 hours, but the impossibility of working a 12-hour limit in the ordinary day led us to adopt a 24-hour limit. One pig succumbed after thirty hours; another after fifty hours had elapsed.

In every instance that we observed the lethal action of tincture of digitalis or of ouabain upon guinea pigs, we noted that the animals made violent ineffectual inspiratory efforts, the phenomena suggesting obstruction of the air passages. In a few minutes the struggles ceased, the animals lost consciousness and were apparently dead, but in every case, the heart could be distinctly felt beating and continued to beat several minutes. On opening the chests of such animals after the heart had ceased beating, the organ was always in diastole. We were convinced from this that the cause of death from poisoning by ouabain was respiratory failure, and to prove this we instituted artificial respiration on six animals, commencing to operate only when voluntary respiration had entirely ceased and the pigs were apparently dead. In all but two instances, marked temporary improvement occurred in the condition of the animals, but we were not able to save any of the series. From this we were led to conclude that death of guinea pigs from poisoning by ouabain is due primarily to respiratory failure but that cardiac poisoning is concerned in this and would ultimately, in itself, cause death. Possibly, by the earlier institution of artificial respiration death could be averted, but we did not consider the point of sufficient importance to justify the necessary infliction of pain.

It must be remembered that the different members ot the digitalis group differ among themselves as regards the relative intensity of their action upon the central nervous system and upon the heart. Possibly, ouabain has more of the direct cardiac action than have the bodies that Nestor used, which would account for our contradictory results.

The following are records of observations typical of the series, save in the two animals where death occurred before commencement of artificial respiration:

No. 1.—1:15 p. m.—Pig weighing 485 grams received .00000035 gm. ouabain per gram body weight, subcutaneously.
2:50 p. m.—Complete cessation of respiration. Animal limp and apparently dead, except for strong, rapid heart beat.
2:52 p. m.—Artificial respiration begun.
2:58 p. m.—Animal struggles feebly; heart beat strong and regular.
3:27 p. m.—Thorax opened. Heart contracting feebly, slow and regular rate.
3:45 p. m.—Heart ceases in systole.

No. 2.—1:45 p. m.—Weight 280 gm. received 0.00000025 gm. ouabain per gm. body weight subcutaneously.
2:30 p. m.—Violent convulsions.
2:35 p. m.—Respiration ceased. Limp and unconscious.
2:38 p. m.—Artificial respiration begun.
2:40 p. m.—Heart beating strongly.
4:00 p. m.—Heart beat faintly felt.
4:05 p. m.—Chest opened; feeble auricular contractions. Ventricles contracted.

An attempt was made to learn with what degree of accuracy the minimum lethal dose of ouabain could be determined upon a series of pigs kept under the same conditions. The following were the results secured:

TABLE No. I.

| Dose per gm. | Survived | Died | Dose per gm. | Survived | Died |
|---|---|---|---|---|---|
| 0.00020 mgm. | 1 | 0 | 0.00030 mgm. | 2 | 6 |
| 0.00023 mgm. | 1 | 0 | 0.00031 mgm. | 0 | 1 |
| 0 00024 mgm. | 1 | 0 | 0.00032 mgm. | 1* | 0 |
| 0.00025 mgm. | 4 | 0 | 0.00033 mgm. | 0 | 1 |
| 0.00026 mgm. | 1 | 1 | 0.00035 mgm. | 0 | 2 |
| 0.00027 mgm. | 4 | 2 | 0.000375 mgm. | 0 | 1 |
| 0 00028 mgm. | 4 | 3 | 0.00010 mgm. | 0 | 1 |
| 0.00029 mgm. | 4 | 5 | | | |

*Died during night over thirty hours after injection.

From this table it appears that 0.00026 mgm. killed one pig, while 0.00032 mgm. failed to kill another within the 24-hour limit, a difference of 23 per cent. It must be understood, however, that the pigs in this series differed in sex, weight, and the locality from which they came. The individual variation was not nearly as large as we had expected to find, and we are inclined to think that even less variation would be encountered when the pigs are of the same age and weight and are grown in the same locality. We regret that the impossibility of getting a sufficient number of animals prevented us from making this series as complete as it should be.

The next point of practical importance was a study of the influence of diet. We were struck by the rapid increase in the weight of those pigs fed liberally and it occurred to us that this rapid increase in weight might cause increase in susceptibility.

Two lots of six pigs each were selected and placed under the same general conditions. One lot was fed liberally on a mixed oat, clover hay, cabbage and carrot diet for seven days. The other lot was fed a restricted amount of the same diet for the same length of time. The well fed pigs gained an average of 21 grams per pig, while those on the restricted diet lost on the average 14 grams per pig. The minimum lethal dose per gram wt. for the pigs on a liberal diet was found to be 0.00026 mg., while that for those on the restricted diet was 0.00025 mg.

TABLE No II.

| No. | Sex | Initial Wt. | Final Wt. | Dose in mgm. | Result of Liberal Mixed Diet |
|---|---|---|---|---|---|
| 1 | M | 328 | 356 | 0.00024 | Survived |
| 2 | M | 241 | 280 | 0.00025 | Survived |
| 3 | M | 678 | 684 | 0.00025 | Survived |
| 4 | M | 173 | 194 | 0.00026 | Died 1 hr. 10 min. |
| 5 | M | 233 | 253 | 0.00027 | Died 1 hr. 20 min. |
| 6 | M | 305 | 319 | 0.00029 | Died 1 hr. 30 min. |
| | | | | | Result of Restricted Mixed Diet |
| 1 | M | 162 | 159 | 0.00024 | Survived |
| 2 | M | 291 | 280 | 0.00025 | Died 1 hr. 50 min. |
| 3 | M | 579 | 531 | 0.00025 | Died 1 hr. 30 min. |
| 4 | F | 229 | 239 | 0.00026 | Died 1 hr. 15 min. |
| 5 | F | 261 | 250 | 0.00027 | Died 1 hr. 15 min. |
| 6 | M | 343 | 321 | 0.00029 | Died 1 hr. 7 min. |

Hunt found variations in diet to influence markedly the susceptibility of guinea pigs to acetonitrile poisoning and suggests that oats diet has some specific action upon the thyroid. It seemed to us that the susceptibility of these animals to digitalis might be similarly influenced, but the impossibility of securing sufficient numbers of pigs and the limited time at our disposal prevented us from securing really conclusive evidence on this point.

Sixteen sound, healthy pigs raised by the same breeder, under similar conditions were divided into two groups of eight. The members of the first group were fed as much oats as they would take and were given a small amount of cabbage once. The members of the second group were fed as much cabbage and carrots as they would take. As may be seen from the table, the whole time of the experiment was fourteen days, and if such results were observed after this limited time on these different diets, more striking differences would be expected when the experiment extends over considerable time. One pig of group one seemed sick and was not used; one pig of group two was discarded because a part of the ouabain solution escaped from the syringe when the injection was being made. (See Table III.)

TABLE No. III.

Series I. Oats Diet

| No. | 7 25–11 Weight | 8–7–11 Weight | 8–8–11 Weight | | |
|---|---|---|---|---|---|
| 1 | 140 | 167 | ... | 0.00000022 | Survived |
| 2 | 200 | ... | 200 | 0.00000022 | Survived |
| 3 | 117 | ... | 116 | 0.00000023 | Survived (Died 50 hrs. after injection) |
| 4 | 174 | ... | 230 | 0.00000024 | Died |
| 5 | 293.5 | ... | 318 | 0.00000024 | Died |
| 6 | 189 | 179 | ... | 0.00000024 | Died |
| 7 | 174 | 175 | ... | 0.00000026 | Died |
| | | | Series II. | Cabbage and Carrots | |
| 1 | 147 | 152 | ... | 0.00000026 | Survived |
| 2 | 315 | ... | 295 | 0.00000026 | Survived |
| 3 | 175 | 178 | ... | 0.00000026 | Survived |
| 4 | 186 | 177 | ... | 0.00000026 | Survived |
| 5 | 221 | ... | 213 | 0.00000026 | Survived |
| 6 | 216 | ... | 215 | 0.00000027 | Survived |
| 7 | 226.5 | ... | 220 | 0.00000028 | Survived |

The results secured by such a limited number of animals are’ of course, not absolutely conclusive. None of the pigs of group two seemed seriously sick, although a dose of 0.00000028 gm. was given one, while pig No. 3 of group two succumbed to 0.00000023 after 50 hours and doses greater than this were invariably fatal within 24 hours. It is unfortunate that the scarcity of guinea pigs and the lack of time prevented us from going more fully into this question. We hope to do this later.

In conclusion, we may say:

1. That the individual variations in the susceptibility of guinea pigs to poisoning by ouabain, as determined in a small series of animals, varies as much as 23 per cent. This may be due to age, weight, or source ot the animals.

2. That the life of a guinea pig that has received a lethal dose of ouabain can be prolonged by the employment of artificial respiration, but in no instance were we able to save the animal.

3. That a diet of oats with a minimum amount of green food seems to cause a decided increase in the susceptibility of guinea pigs; while a diet exclusively of green food seems to diminish susceptibility.

The first and third points are of great practical importance, and, if they be confirmed, would seem to render the guinea pig method as now employed unsuited for standardization of the members of the digitalis group.—*Department of Experimental Medicine.*

# A STUDY OF THE BROMINE AND IODOMETRIC METHODS FOR THE DETERMINATION OF RESORCINOL.*

BY C. M. PENCE, B. S.

[*Paper read before the Pharmaceutical Division, Indianapolis meeting, Amer. Chem. Soc., June, 1911, and reprinted from the Journal of Industrial and Engineering Chemistry, Vol. 3, No. 11, November, 1911.]

Koppeschaar's (1) modification of Landolt's bromine method for the determination of phenol suggested several years ago that other phenols (2, 3 and 4) might be estimated by practically the same procedure.

Degener (5) devised the following bromine method for resorcinol: A standard solution of free bromine is run into the resorcinol solution as long as a precipitate forms and until solution becomes yellow. Then potassium iodide is added and the liberated iodine titrated with $N/10$ thiosulphate.

Richards (6) proposed an iodometric method similar in procedure to the bromine method of Degener with the exception that it is necessary to add some substance like sodium acetate to cause greater completion of the reaction by uniting with the hydriodic acid formed during the substitution.

A study was made of these two methods with the purpose of finding a rapid and yet accurate method for the estimation of resorcinol.

Resorcinol of uncorrected m. p. 109-110° C. was recrystallized from distilled water, the crystals were disintegrated and allowed to drain thoroughly, then recrystallized from absolute alcohol free from aldehydes, etc., The crystals thus obtained were broken apart, thoroughly drained, desiccated in vacuum desiccator over sulphuric acid, powdered and redesiccated to free from the last traces of alcohol.

Volumetric solutions were prepared from these crystals and an attempt was made to apply the Koppeschaar procedure for the

| Expt. | Resorcinol. cc. | Br $N/10$. cc. | $H_2O$. cc. | HCl. cc. | Time standing | $H_2O$. cc. | KI 20 per cent. cc. | Time standing | $H_2O$ cc. | Procedure | Time Standing | $CHCl_3$. cc. | Result per cent. | Br $N/10$ Excess |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 20 | 50 | 50 | 5 | 30 min. | .... | 10 | ...... | .... | .......... | ...... | 5 | 102.58 | .......... |
| 2 | 20 | 50 | 30 | 5 | 60 " | 200 | 5 | 10 min. | .... | .......... | ...... | .. | 102.6 | .......... |
| 3 | 20 | 50 | 30 | 5 | 5 " | .... | 10 | ...... | 200 | .......... | ...... | 20 | 100.13 | .......... |
| 4 | 20 | 50 | 30 | 5 | 5 " | 200 | 10 | ...... | .... | .......... | ...... | .. | 100.15 | .......... |
| 5 | 20 | 50 | 30 | 5 | 5 " | .... | 5 | ...... | 200 | .......... | ...... | . | 100.1 | .......... |
| 6 | 10 | 20 | .. | 5 | 1 " | .... | 10 | 1 hr. | 150 | .......... | ...... | 10 | 100.3 | 1.5 |
| 7 | 10 | 20 | .. | 5 | 1 " | .... | 10 | 2 hrs. | 150 | .......... | ...... | 10 | 99.9 | 1.7 |
| 8 | 20 | 50 | 20 | 5 | 1 " | .... | 10 | 2 " | 150 | .......... | ...... | 10 | 99.78 | .......... |
| 9 | 20 | 50 | 20 | 5 | 1 " | .... | 10 | 2 " | 150 | .......... | ...... | 10 | 99.71 | .......... |
| 10 | 50 | 40 | 10 | 5 | 1 " | .... | 10 | 1 hr. | 150 | .......... | ...... | 10 | 99.89 | 8.25 |
| 11 | 50 | 40 | 10 | 5 | 1 " | .... | 10 | 1 " | 150 | .......... | ...... | 10 | 99.94 | 8.22 |
| 12 | 20 | 20 | 20 | 5 | 1 " | .... | 10 | 30 min. | 150 | .......... | ...... | 10 | 100.39 | 1.42 |
| 13 | 20 | 20 | 20 | 5 | 1 " | .... | 10 | 30 min. 40°C | 150 | .......... | ...... | 10 | 99.82 | 1.6 |
| 14 | 20 | 20 | 20 | 5 | 1 " | .... | 10 | 1 hr. | 150 | .......... | ...... | 10 | 99.99 | 1.48 |
| 15 | 20 | 50 | 30 | 5 | 1 " | .... | 10 | 1 " | 250 | Shook well | 1 min. | .. | 99.4 | about 12.0 cc. |
| 16 | 20 | 50 | 30 | 5 | 1 " | .... | 10 | ...... | 250 | " | 1 " | .. | 99.3 | " |
| 17 | 20 | 50 | 30 | 5 | 1 " | .... | 15 | ...... | 250 | " | 5 " | .. | 99.41 | " |
| 18 | 20 | 50 | 30 | 5 | 1 " | .... | 15 | ...... | 250 | " | 1 hr. | .. | 99.4 | " |
| 19 | 20 | 50 | 30 | 5 | 1 " | .... | 10 | 1 hr. | 200 | " | 1 min. | .. | 99.4 | " |
| 20 | 20 | 50 | 30 | 5 | 2 " | .... | 10 | 1 " | 200 | " | 1 " | 20 | 99.5 | " |
| 21 | 20 | 50 | 30 | 5 | 5 " | .... | 10 | 5 min. | 200 | " | 2 " | 20 | 99.6 | " |
| 22 | 20 | 50 | 30 | 5 | 5 " | .... | 10 | 5 " | 200 | " | 2 " | 20 | 99.56 | " |
| 23 | 20 | 50 | 30 | 5 | 5 " | .... | 10 | 5 " | 200 | " | 2 " | .. | 99.59 | " |
| 24 | 20 | 50 | 30 | 5 | 2 " | .... | 10 | 1 hr. | 200 | " | 1 " | 20 | 99.5 | |
| 25 | 20 | 50 | 30 | 5 | 1 " | .... | 10 | 2 min. | 200 | " | ...... | 20 | 99.76 | " |
| 26 | 20 | 50 | 30 | 5 | 1 " | .... | 10 | 2 " | 200 | " | ...... | 20 | 99.74 | " |
| 27 | 20 | 50 | 30 | 5 | 1 " | .... | 10 | 1 " | 200 | " | ...... | 20 | 99.74 | " |
| 28 | 20 | 50 | 30 | 5 | 1 " | .... | 10 | 1 " | 200 | " | ...... | 20 | 99.76 | " |
| 29 | 20 | 50 | 30 | 5 | 5 " | .... | 5 | 1 hr. | 200 | " | ...... | .. | 99.30 | " |
| 30 | 25 | 50 | 50 | 5 | 1 " | .... | 5 | 1 " | 200 | " | ...... | .. | 99.25 | " |
| 31 | 25 | 50 | 50 | 5 | 1 " | 200 | 5 | ...... | .... | " | 20 " | .. | 99.95 | " |
| 32 | 20 | 50 | 50 | 5 | 2 " | 200 | 5 | ...... | .... | " | 15 " | .. | 99.92 | " |
| 33 | 20 | 50 | 30 | 5 | 2 " | 200 | 5 | ...... | .... | .......... | 30 " | .. | 99.92 | " |
| 34 | 25 | 50 | 50 | 5 | 1 " | 200 | 5 | 1 hr. | .... | .......... | ...... | .. | 99.49 | " |
| 35 | 25 | 50 | 50 | 5 | 1 " | 200 | 5 | 1 " | .... | .......... | ...... | .. | 99.45 | " |
| 36 | 20 | 50 | 30 | 5 | 2 " | .... | 5 | 3 min. | 200 | Shook well | 1 min. | .. | 100.00 | " |
| 37 | 20 | 50 | 30 | 5 | 5 " | .... | 5 | 6 " | 200 | " | 1 " | .. | 100.00 | " |
| 38 | 20 | 50 | 30 | 5 | 5 " | .... | 5 | 5 " | 200 | " | 1 " | .. | 99.96 | " |
| 39 | 20 | 50 | 30 | 5 | 5 " | .... | 5 | 3 " | 200 | " | 1 " | .. | 99.[illegible]6 | |

| Expt. | Resorcinol. cc. | Br N/10. cc. | $H_2O$. cc. | HCl. cc. | Time standing | $H_2O$. cc. | KI 20 per cent. cc. | Time standing | $H_2O$. cc. | Procedure | Time Standing | $CHCl_3$. cc. | Result per cent. | Br N/10 Excess |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 40 | 20 | 50 | 30 | 5 | 5 min. | .... | 5 | 5 min. | 200 | Shook well | 1 min. | .. | 100.0 | about 12.0 cc. |
| 41 | 20 | 50 | 30 | 5 | 1 " | .... | 10 | ...... | 200 | " | ...... | 20 | 99 86 | " |
| 42 | 20 | 50 | 30 | 5 | 2 " | .... | 10 | ...... | 200 | " | ...... | 20 | 100.05 | " |
| 43 | 20 | 50 | 30 | 5 | 1 " | .... | 5 | ...... | 200 | " | ...... | .. | 100.1 | " |
| 44 | 20 | 50 | 30 | 5 | 3 " | .... | 5 | ...... | 200 | " | 1 min. | .. | 100.21 | " |
| 45 | 20 | 50 | 30 | 5 | 5 " | .... | 5 | ...... | 200 | " | 1 " | .. | 100.4 | " |
| 46 | 20 | 50 | 30 | 5 | 1 " | 200 | 5 | ...... | .... | " | 10 " | .. | 100.03 | " |
| 47 | 20 | 50 | 30 | 5 | 30 " | 200 | 5 | ...... | .... | " | 45 " | .. | 99.93 | " |
| 48 | 20 | 50 | 30 | 5 | 30 " | 200 | 5 | ...... | .... | " | 15 " | .. | 100.1 | " |
| 49 | 20 | 50 | 30 | 5 | 45 " | 200 | 5 | ...... | .... | " | 1 hr. | .. | 100.1 | " |
| 50 | 20 | 50 | 30 | 5 | 15 sec. | 200 | 5 | ...... | .... | " | 1 min. | .. | 99.7 | " |
| 51 | 25 | 50 | 30 | 5 | 1 min. | 200 | 5 | ...... | .... | " | 1 " | .. | 101.04 | 34.6 cc. |
| 52 | 25 | 50 | 30 | 5 | 1 " | 200 | 5 | ...... | .... | " | 3 " | .. | 100 26 | 34.9 cc. |
| 53 | 20 | 50 | 30 | (10 cc. 10 %) | 1 " | 200 | 5 | 1 min. | .... | .......... | ...... | .. | 99 86 | .......... |
| 54 | 25 | 50 | 50 | 5 | 1 " | 200 | 5 | ...... | .... | Shook well | 1 min. | .. | 99.95 | about 16 0 cc. |
| 55 | 25 | 50 | 50 | 5 | 1 " | 200 | 5 | ...... | .... | " | 3 " | .. | 99.95 | " |
| 56 | 25 | 50 | 50 | 5 | 1 " | 200 | 5 | ...... | .... | " | 8 " | .. | 99.97 | " |
| 57 | 25 | 50 | 50 | 5 | 1 " | 200 | 5 | ...... | .... | " | 14 " | .. | 99 95 | " |
| 58 | 25 | 50 | 50 | 5 | 1 " | 200 | 5 | ...... | .... | " | 5 " | .. | 100 00 | " |
| 59 | 25 | 50 | 50 | 5 | 1 " | 200 | 5 | ...... | .... | " | 8 " | .. | 100.12 | " |
| 60 | 25 | 50 | 50 | 5 | 1 " | 200 | 5 | ...... | .... | " | 12 " | .. | 100.04 | " |
| 61 | 25 | 50 | 50 | 5 | 1 " | 200 | 5 | ...... | .... | " | 15 " | .. | 100.04 | " |
| 62 | 25 | 50 | 50 | 5 | 1 " | 200 | 5 | ...... | .... | " | 20 " | .. | 99.99 | " |

| Exp. | Resorcinol cc. | N/10 I. cc. | $NaCH_3COO$. Grams. | $Na_2HPO_4$. Grams. | $NaHCO_3$. Grams. | Time standing | $H_2O$ added cc. | Result | Result, after excess thiosulphate titrated back | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| 63 | 20 | 50 | .. | 2 | .... | 5 min. | 200 | ...... | ...... | Too dark to titrate |
| 64 | 20 | 50 | 1 | .. | .... | 1 hr. | 200 | 99 2 | 98 94 | |
| 65 | 20 | 50 | 1 | .. | .... | 15 min. | 200 | 98 6 | 98.35 | |
| 66 | 20 | 50 | 1 | .. | .... | at once | 200 | 98 4 | 98 7 | |
| 67 | 20 | 50 | 1 | .. | .... | 30 min. | 200 | 99.33 | 99.15 | |
| 68 | 20 | 50 | 1 | .. | .... | at once | 200 | 99 33 | 99.15 | |
| 69 | 20 | 50 | 5 | .. | .... | at once | 200 | 97 7 | 97 5 | |
| 70 | 20 | 50 | 2 | .. | .... | at once | 200 | 98.2 | 98.3 | |
| 71 | 20 | 50 | 5 | .. | .... | 3 hrs. | 200 | ...... | ...... | Too dark to titrate |
| 72 | 20 | 50 | .. | .. | 0 5 | 5 min. | 200 | ...... | ...... | Too dark to titrate |
| 73 | 20 | 50 | .. | .. | 2.0 | 3 hrs. | 200 | ...... | ...... | Too dark to titrate |

volumetric estimation of phenol as outlined in the U. S. Pharmacopœia, 1900. This method seemed to have several advantages over that of Degener inasmuch as the Koppeschaar bromine solution is much superior to a standard solution of free bromine in stability and ease of handling.

The U. S. P. or Koppeschaar method is briefly as follows: The aqueous phenol solution is measured with a pipette into a 500 cc. receptacle with ground glass stopper. A measured quantity of standard bromine solution sufficient to give a small excess of bromine is added, then 5 cc. of concentrated HCl. The bottle is shaken and let set 30 minutes. Five cc. of 20 per cent. KI are added and the bottle shaken. One cc. of $CHCl_3$ is added, bottle again shaken and free iodine titrated with $N/10$ thiosulphate until chloroform is free from color. Starch was used as an indicator instead of chloroform, which was added merely as a solvent for precipitated phenols, thus increasing the sensitiveness of end point.

It was found that when solution was allowed to stand 30 minutes after addition of HCl the results would be much too high. This was attributed to the formation of tribromresorcinolbrom (7) which did not readily decompose after the addition of KI. Expts. 1 and 2 show this tendency,Expt. 2 also showing that short time of standing after addition of KI did not permit the decomposition of the additive compound formed.

An attempt was now made to counteract tendency for formation of this compound by shortening the reaction period of bromine upon resorcinol. Expts. 3, 4 and 5 show much lower results.

In the next experiments (6-14) the reaction period of bromine was kept at 1 min. and solution was allowed to stand after the addition of KI. It was found that KI caused a reversal whereby the tribromresorcinolbrom was entirely decomposed. Expts. 6-12 and 14 show this reversal effect of KI and length of time required for complete reversal under the specific experimental conditions used. Expt. 13 shows that heat increases the speed of reversal.

Now it was observed during the course of the above experiments that a pronounced precipitate formed upon the addition of KI. It was thought probable that this precipitate consisted partially of or occluded some tribromresorcinolbrom and prevented a ready reversal. This supposition was verified by Expts. 15-28. By proper dilution the precipitation was prevented. However, it was found that reversal did not cease after breaking down the tribromresorcinolbrom but that some of the tribromresorcinol was decomposed. Chloroform was used, thinking that decomposition might be taking place almost entirely during the titration. This was found to be untrue (Expts. 20, 24, 25, 26, 27, 28). Nevertheless, these experiments did show that amount of reversal was dependent upon time of standing after dilution. Compare Expts. 15-24 with Expts. 25-28.

It now remained to ascertain whether or not a smaller amount of KI would counteract tendency for decomposition of tribromresorcinol and under what conditions such would obtain. It was found that this decomposition was prevented; that solution must not stand too long after addition of KI before dilution; that in diluted condition, time of standing might be somewhat prolonged without deleterious results; that after dilution and addition of KI, solution should not stand as long as 1 hour before titration (Expts. 29, 30-31, 32, 33-34, 35).

Several experiments were run varying the length of time bromine was allowed to react with resorcinol in order to study the length of time it would take KI to produce proper reversal under varying conditions. In general, it may be said that KI should react at least as long as bromine is permitted to react with resorcinol. Experiments 36-49.

It was found in Expt. 50 that reaction between bromine and resorcinol would not be completed in 15 secs.; in Expts. 51 and 52 that solution should set at least 5 mins. after addition of KI when excess of bromine is large; in Expt. 53 that reaction between bromine and resorcinol solutions is much more rapid when strong acid is used.

The method of Degener would be open to considerable error, due to formation of the additive compound and to insufficient time and dilution for its decomposition.

Richards' iodometric method has several features that condemn it: 1st, solutions darken upon standing so that end point is obscured; 2nd, even upon prolonged standing results are much too low (Expts. 63-73).

The following method is suggested for the estimation of resorcinol in commercial resorcinol: Its accuracy may be noted by reference to Expts. 54-62.

Weigh out 1.4563 g. resorcinol. Dissolve in distilled water, filter if necessary, and dilute to 500 cc. in a volumetric flask. Withdraw a 25 cc. portion with a pipette and place in a 500 cc. receptacle with ground glass stopper. Add 50 cc. *N*/10 bromine solution and 50 cc. dist. water. Add 5 cc. conc. HCl, shake and let set about 1 minute. Dilute with about 200 cc. water, add 5 cc. 20 per cent. KI, shake vigorously and let set about 5 minutes. Titrate, using starch as indicator. Divide number of cc. of *N* 10 bromine solution consumed by 0.4 (or multiply by 2.5) for correct percentage of resorcinol.

A blank should be run along with the determination, using the same pipette and draining in the same manner in order to obtain an exact duplicate titre of *N*/10 bromine solution. 7-10 cc. of 20 per cent KI should be used instead of 5 cc. as in regular determination.

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# THE IMPROVEMENT OF MEDICINAL PLANTS

F. A. MILLER, B. S.

[Read before the Botanical Section of the Indiana Academy of Science, November, 1911].

The principles of plant breeding have as yet hardly been brought to bear upon the improvement of medicinal plants. The necessity of improvement and the possibilities of the application of modern methods of breeding to this group of plants has led the writer to undertake a series of investigations upon this subject. A discussion of the results and progress of these investigations is the object of the present paper.

The broadening influence of plant breeding is gradually bringing under control, members from all groups of the plant kingdom, and it is only fitting that this very important group of plants should be made to yield the best of nature's possibilities. That the group contains many plastic forms which will yield readily to modern methods of breeding is evidenced by the fact that some of the most potent medicinal forms appear in families from which have been obtained many valuable horticultural varieties. The Solonaceæ for example, with the genera Atropa, Hyoscyamus, Datura, Solanum and Capsicum and the no less important Scrophulariaceæ containing the now widely known genus Digitalis which is found to be equally as variable both in chemical and physical characteristics as the common garden forms derived from the same and related genera, will serve to illustrate this point.

A review of the literature on drug plant improvement reveals but few attempts at systematic investigation by the employment of standard methods of breeding. On the other hand much has been written and no little accomplished upon the introduction and cultivation of medicinal plants. Introduction and cultivation with no improvement has been the order of procedure. It is quite true that some improvement has resulted from a changed environment and a reduced struggle for existence but not through the intensive application of particular methods. Improvement by means of seed and plant selection, the isolation and testing of favorable varieties, the study of soil and climatic conditions, trials in hybridization, grafting or other methods which might prove applicable, have not been tried except in comparatively few instances. Had the government made selections of hydrastis (Hydrastis canadensis L.) ten years ago upon a basis of alkaloidal percentage, their plantings made at that time might now be yielding interesting and valuable data upon the behavior of this plant under cultivation. Problems relating to propagation, cultivation, collecting and curing have been solved, but the cause of the wide range in the percentage of alkaloids in this drug remains an unknown factor. From Jan. 6, 1909 to Nov. 8, 1911 this range in percentage of hydrastine was found to be from 2.79 to 7.60.

Another illustration of cultivation without improvement which will at the same time serve to demonstrate the practical value of the application of a single standard method is the growing of the drug burdock. This drug consists of the root of Arctium lappa L. collected from plants of the first year's growth. For the past fifteen years this plant has been grown under cultivation on a commercial scale near Indianapolis for the production of the first year's roots in the recent condition. The superior quality of the resulting product over that obtained from wild plants was early recognized. The drug was more uniform in every respect, almost free from fibrous tissue and is believed to produce a more active preparation. With this favorable beginning it is surprising, indeed, to learn that the final results of fifteen or more years of continuous cultivation have failed to advance this plant beyond the point reached at the end of the first year. Upon seeking an explanation of this fact it was found that from one year to the next the total seed supply came from wild plants found growing by the roadside. This plant being a biennial and the crop being harvested at the end of the first year's growth has left the farmer at the end of each season to search for a new seed supply. When interviewed as to why seed plants were not selected upon a basis of green weight of root produced the answer has been that it would not pay. That seed and plant selection could be made to pay can be demonstrated upon a basis of original investigation and reliable data. A study of the results obtained in the Division of Botany of the United States Department of Agriculture upon "The Superior Value of Large, Heavy Seed," indicates an increase in the weight of the plant which is in direct proportion to the weight of the seed employed. To obtain data for calculations upon such a basis, burdock seeds were taken from a lot collected miscellaneously from wild plants and separated into light and heavy portions. The separation was accomplished by the use of an apparatus designed after one used for the separation of tobacco seeds, in the experiments on tobacco breeding performed at the Connecticut State Experiment Station. This apparatus is described in the Yearbook of the Department of Agriculture for 1904. Slight modifications were found necessary to adapt it to this type of seed. A number of seeds from both the heavy and light portions were accurately weighed. The average of the light seeds was 0.0035 grams and of the heavy 0.0084, a ratio of 1:2.40. The variation in the heavy seeds was from 0.0077 to 0.0091 grams and in the light from 0.0017 to 0.0046. The greater variation in the light seeds was found to be due to the force of the air current employed in the separation. That a separation into light, medium and heavy can be made just as readily as into light and heavy will be shown in connection with another form.

A planting of three acres of burdock of the present year has been chosen as a type upon which to calculate the increase in yield which might have been obtained by means of seed selection. This planting was made upon a deep mellow loam and the total yield of 33,890 pounds is rather unusual. Assuming that the seed supply used on this planting consisted of light and heavy seeds in the determined proportion of 1:2, the total yield can be theoretically divided into two portions of 6,778 and 27,112 pounds, respectively produced by the light and heavy seed. Had the light seed used been equal in weight to the heavy seed they would have produced twice as much as they theoretically did which would have been 13,556 pounds. This would make a total yield of 40,668 pounds instead of 33,890, an increase of 20 per cent.

Even of greater importance, however, to the medical and pharmaceutical professions is the improvement of henbane and digitalis, representing as they do two valuable drugs of the United States Pharmacopœia.

By their being more amenable to chemical and physiological methods of standardization, the investigator is furnished with additional means of following the progress of various methods of improvement. Official henbane is supposed to consist of the dried leaves and flowering tops of Hyoscyamus niger L. collected from plants of the second year's growth and yielding not less than 0.08 per cent. of mydriatic alkaloids. That the above chemical and botanical conditions of this drug rarely obtain has been clearly demonstrated. An average of a large number of samples examined from August 25, 1908 to September 23, 1911 indicates that only 13 per cent. conformed to the requirements of the United States Pharmacopœia from a chemical standpoint. The average alkaloidal percentage of all samples for the same period was 0.0640 and the range of variation in individual samples was from 0.018 to 0.125 per cent.

The botanical characteristics approach the official requirements with no greater degree of certainty. The crude drug varies from fragmentary specimens of unevenly cured leaves and stems containing an admixture of grass, straw and other plant parts, as well as the refuse of chicken and barn lots, to pure, bright, clean, evenly cured leaves, compressed in such a manner that the entire leaf is available for inspection. The botanical source of the drug is also questionable as evidenced by numerous seed tests from samples and lots from which viable seeds could be obtained. These tests have shown this drug to consist of a mixture of two distinct forms, the so called annual variety which is not included in the pharmacopœial description appeared regularly in these tests. Specimens of this form have been grown to maturity in the writer's garden and close observation has failed to substantiate the statements that it is identical with Hyoscyamus niger L. Seedling plants from both forms are now under observation and will be studied both botanically and chemically through succeeding generations. To determine the possibility of obtaining a uniform henbane, seeds were purchased from August and George Fischer of London which were found to possess a high percentage germination. Plants from these seeds differed greatly in size and vigor in the early seedling stages, and a selection from approximately two thousand seedlings was necessary in order to obtain one hundred and forty plants of uniform character. The great variation noted in the seedling stages of these plants led to a second application of the seed separator. The seeds of this form being quite small, several portions of two hundred seeds each, were taken from light, medium and heavy separations. The respective weights of these were 0.0505, 0.0945 and 0.1250 grams. Plantings from these different weight seeds have not progressed sufficiently at the present time to justify a discussion of the merits of the methods. Open field experiments with this drug have demonstrated that seed germination is uncertain, that the plants are subject to the attacks of many insects and that the seedlings transplant with considerable difficulty. No conclusions can be drawn from these facts, since the seeds employed in all out door tests were imported and but very few lots of such seeds have given satisfactory results. The two forms of henbane mentioned i. e. annual and biennial do not represent the limit of possibilities in this genus. According to Engler and Prantl the genus consists of eight species widely distributed throughout temperate and subtropical regions. In the subtropical is found the very promising form, Hyoscyamus muticus L., yielding over one per cent. of alkaloids, while the remainder are found in the temperate regions. Of these, some have passed through periods of prominence in different countries, as Hyoscyamus albus in France, the annual form in parts of Germany and the biennial form in England, all of which suggest the possibilities within the entire genus.

In this group of plants the necessity of systematic efforts leading to the development of pure bred strains of promising species, and to an increase in the percentage of alkaloids is indicated by the above data. The famous corn breeding experiment at the Illinois State Experiment Station, the records of which now cover thirteen generations, indicates what may be done through selection. That the efforts to increase and decrease the protein and oil have met with great response is shown by the fact that two strains of corn have been produced out of a single variety, one of which contains more than half again as much protein as the other. The effect upon the oil content is even more striking since from this same variety two other strains have been produced one of which contains practically three times as much oil as the other. The sugar beet industry of this and other countries is illustrative of the necessity of the intensive breeding, essential to the production of high yielding plants. The gain of 22.2 per cent. in the total sugar beet output of Germany for 1910-11 with an increased acreage of only 3.6 per cent. was due to three factors one of which was the higher sugar content of the beets. Experiments have shown a variation of seven per cent. in sugar content, in beets of the same percentage grown in different localities, a fact which is suggestive of the necessity of a careful choice of localities for drug plant investigation. The introduction of various species of Cinchona into India by the British government over fifty years ago has long passed the experimental stage, but the records of the difficulties overcome, stand as convincing evidence of what may be accomplished in the introduction and improvement of arborescent forms. Many of these forms were long grown and propagated under glass until individuals could be isolated which could endure the new environment and be made to serve as starting points for future generations. The work of the Department of Agriculture on capsicum, camphor, tea, tobacco and a host of other forms bears out the evidence of the foregoing, and furnishes the worker with a wealth of data applicable in many ways to drug plant improvement.

The drug digitalis, consisting of the dried leaves of Digitalis purpurea L. collected from the second year's growth at the commencement of flowering, is equally in need of improvement.

Physiological tests have shown a variation in the toxicity of preparations made from drug representing different geographical sources. Differences of opinion also exist as to the relative medicinal value of first and second year leaves, of those from wild and cultivated plants and of those from plants of different species and varieties, as well as to the effects of different periods of collecting and methods of curing, packing and storing of the crude drug and the relative value of preparations made from fresh and dry leaves. A botanical examination of the genus reveals conditions which will probably account in part at least, for the above differences of opinion.

The genus is a large one consisting of twenty-one widely distributed species, a fact which alone increases the possibilities of an admixture of leaves from several species or from the same species growing under different climatic conditions. This possibility is also increased by the numerous varieties originated by florists and gardeners who have not been slow in recognizing the æsthetic value of the genus. Their catalogues now contain many standard varieties which are noted for their attractive nature and ease of culture. The official species Digitalis purpurea L. has figured largely in this production, having given rise to no less than half a dozen distinct forms which are now listed as hardy perennials. Other species have been active in this respect but have not produced such a diversity of forms. This property of a genus to yield multiple forms is strongly suggestive of a wide range of variations in the corresponding percentage of active principles. The botanical inspection of the crude drug is in no degree indicative of this percentage of active principle but that such an indication is possible is suggested by recent investigations of Gregory upon the association of transmissable characters in Primula sinensis where it has been shown that some characters are always found to accompany others with recurring regularity. But until such a convenient relation is found to exist between active principle and specific morphological characters, the botanical examination can only point to the possible source and not to the comparative medicinal value of the drug. Observations, now being made upon several species and varieties of digitalis, have revealed variations which would prove of considerable commercial value if found associated with a correspondingly high percentage of active principle. A dissimilarity of leaf forms has been obtained in plants grown from seed which offer valuable material for selection as to form, size, color and arrangement, relative number per plant, length of petiole, texture and curing properties. Differences have also been noted as to rate and percentage of seed germination, flowering period, production of suckers, hardiness and ease of propagation and cultivation.

In the course of an investigation upon a form of digitalis found growing adventively in parts of Oregon, an excellent example has been obtained of the uncertainty of the botanical origin of commercial digitalis and the difficulty of the separation of distinct forms upon a basis of leaf characters. One hundred and forty plants said to represent the first year's growth were obtained from this source. These plants were collected in the open and represented a locality from which commercial digitalis leaves had been marketed. These plants arrived in excellent condition and were transplanted in the open near Indianapolis in early spring. They were closely observed throughout the season and during this time but few plants flowered, all of these, however, coming true to the description of Digitalis purpurea L. and were quite uniform as to leaf characters. The plants made excellent growth during the summer and went into the winter as large, strong, healthy plants in fit condition for experimental purposes. To test this form for hardiness in this latitude these plants were left in an exposed locality in an unprotected condition throughout the year. Forty-three per cent. withstood the severe winter of 1910-11 and flowered, but very irregularly during the ensuing summer. The effects of the exposure were manifested by a much lower production of leaves than that attained during the first year's growth. Among the sixty plants which survived the winter there was one which produced racemes of pure white flowers instead of the characteristic purple flowers of Digitalis purpurea L. The presence of this form among a comparatively small number of plants indicates the admixture of a varietal form, the medicinal properties of which are not known. The other individuals which flowered were fairly uniform in all visible characters except as to variations in flower arrangement, some bearing upright instead of drooping flowers, an arrangement which gave the plants a striking appearance. Seed selections were made from these forms and a further study will be made of them both for hardiness and as to fitness for medicinal use.

Preliminary work has been done on seed selection with several forms of digitalis. Seed tests of light and heavy seeds obtained by means of the apparatus previously mentioned have given striking results in the early stages of seedling growth as shown by the accompanying photographs. The following data will indicate the accuracy of this method of seed selection and the uniformity in the seeds separated. It also demonstrates the practical value of the method if applied to the commercial production of digitalis leaves. Seeds collected from the foregoing plants of Digitalis purpurea L. were separated into light, medium and heavy. The extreme smallness of these seeds made it necessary to use five hundred seeds from each separation for weighings. Seeds of Digitalis grandiflora Lam. obtained from Henry A. Dreer of Philadelphia were also separated, weighed and tested. These were heavy enough to be weighed in one hundred lots and were of such uniformity that they were only separated into light and heavy portions.

The following table includes the results of these separations and weighings:

### DIGITALIS PURPUREA

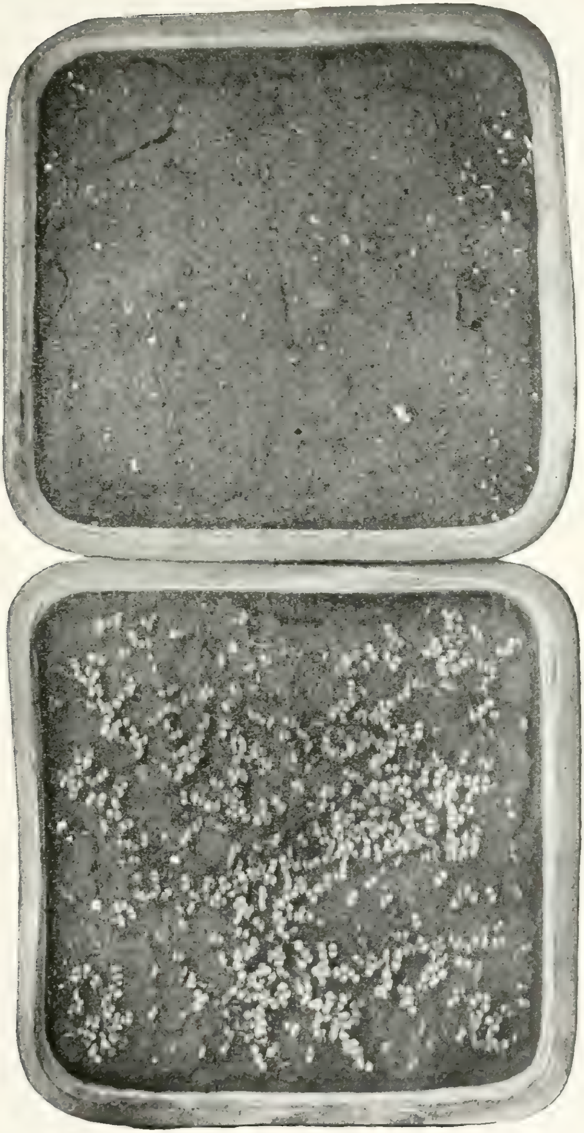
| LIGHT— | MEDIUM | HEAVY— |
|---|---|---|
| 500 Seeds | 500 Seeds | 500 Seeds |
| 0.0217 | 0.0253 | 0.0341 |

### DIGITALIS GRANDIFLORA

| LIGHT | | HEAVY | |
|---|---|---|---|
| 100 Seeds | 0.0168 gm. | 100 Seeds | 0.0215 gm. |
| 100 Seeds | 0.0167 gm. | 100 Seeds | 0.0223 gm. |
| 100 Seeds | 0.0161 gm. | 100 Seeds | 0.0223 gm. |
| 100 Seeds | 0.0161 gm. | 100 Seeds | 0.0215 gm. |
| 100 Seeds | 0.0164 gm. | 100 Seeds | 0.0217 gm. |
| Total | 0.8821 | Total | 0.1093 |

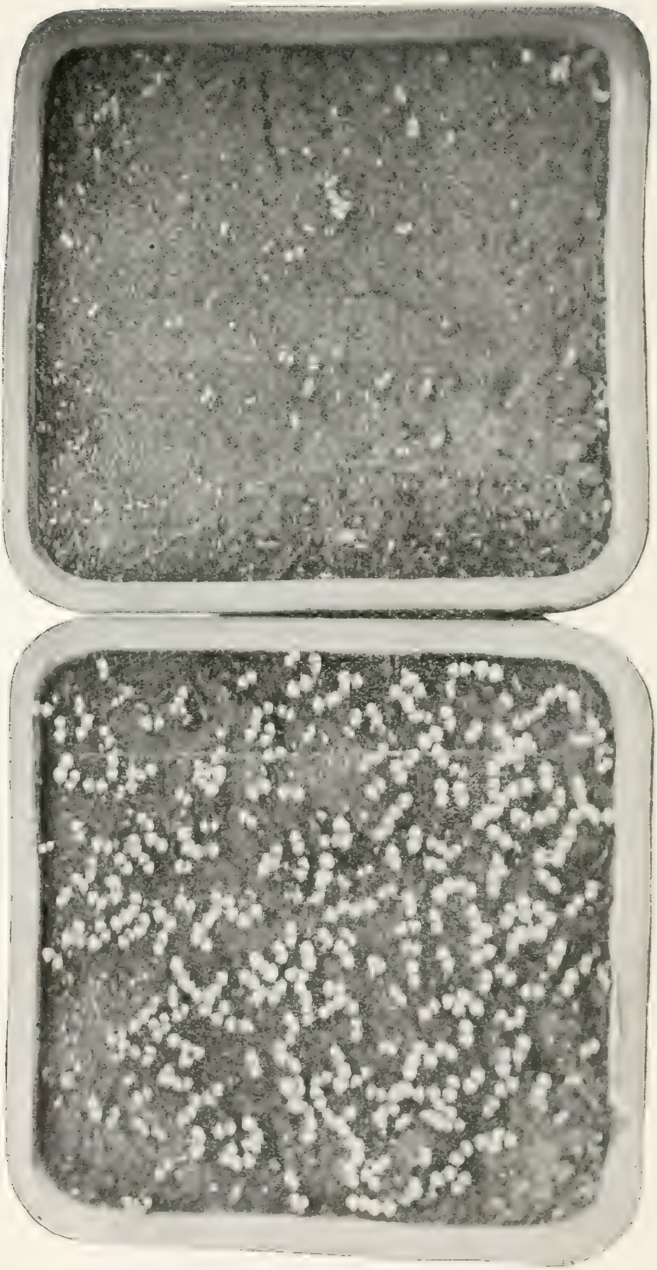
In conclusion it is only necessary to say that the application of these methods of breeding and the possibilities in drug plant improvement herein suggested should be extended until they include such valuable forms as cannabis indica, belladonna, buchu and others. Hardy productive varieties of these forms must be discovered or produced and these brought under the influence of modern agriculture where they may be utilized to meet the conditions of growing scarcity, advancing prices and the demand for better products.

*Botanical Department.*



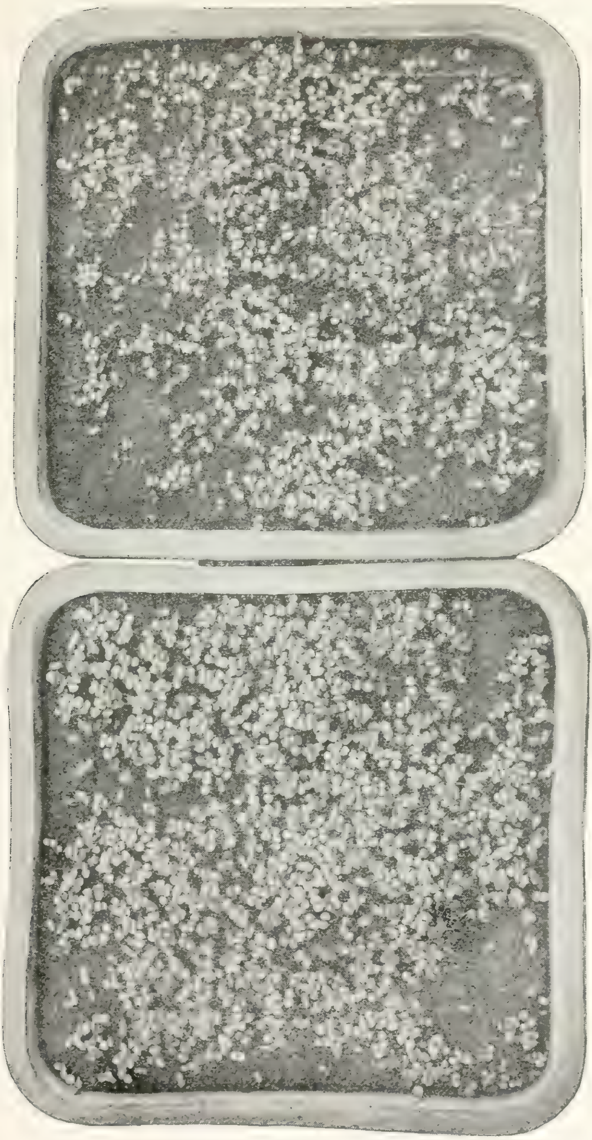
No. I.—DIGITALIS PURPUREA L.

Upper seed pan sown with light seed. Lower seed pan sown with heavy seed. Equal parts (by weight) of light and heavy seeds were sown



No. II.—DIGITALIS AMBIGUA MURR.

Upper seed pan sown with light seed. Lower seed pan sown with heavy seed. Equal parts (by weight) of light and heavy seeds were used



No. III.—DIGITALIS PURPUREA L.

Results obtained from selected and non-selected seeds. Upper seed pan sown with non-selected seed. Lower seed pan sown with selected heavy seed.

# THE RELATIVE STRENGTH OF FRESH AND OLD SAMPLES OF THE FLUID EXTRACT OF ERGOT

CHAS. C. HASKELL AND CHAS. R. ECKLER

The importance of having medicinal preparations of constant strength can not be overestimated. Many factors over which the physician has no control of necessity arise when patients are treated with drugs, and if the drugs administered are of unknown and variable strength, truly rational therapy is impossible. Fortunately, chemical investigation has been so fruitful of results that most of the important drugs used in therapeutics can now be standardized with great accuracy.

Even if preparations are of definite strength at the time of manufacture, however, the question arises as to whether deterioration can not occur before their employment. It is well known that in aqueous solutions some of the alkaloids rapidly decompose. Careful examination (18) has shown, however, that most galenical preparations which are susceptible of chemical assay retain their strength undiminished for a number of years.

Unfortunately, there is a group of drugs for which no chemical assay method is commercially practicable. Most important of this group are the cardiac tonics while ergot is of scarcely less moment.

Since ergot can not be standardized by chemical means, it is easy to understand why attempts have been made to estimate its strength by observation of the effect of the drug upon lower animals. The question of deterioration being so obviously dependent upon standardization, it seems necessary to consider briefly the three methods commonly employed in the attempt to produce preparations of uniform strength.

Practically all recent investigators have observed that fresh ergot preparations have a distinct pressor activity. Dixon, (6) apparently, was the first to utilize this action to measure the therapeutic efficiency of the drug. He states: "I have found, however, that rise in blood pressure in mammals is proportional to the effect upon the uterus." He advocates the use of rabbits, injecting the drug into the femoral vein and recording the carotid blood pressure by means of a mercury manometer.

While Dixon's paper was very brief and incomplete, Wood and

Hofer (20) have studied the pressor activity of a number of commercial samples of ergot with great thoroughness. They, too, consider that the actions upon the blood pressure and upon the uterus are parallel. They believe that: "There can be little doubt but that the increased contractions of the uterus and the vasomotor stimulation are part of a widespread effect of the drug, involving all involuntary muscles. * * * and the changes in the circulations, in the intestines, and in the uterus are but a part of one general action." This being the case, they maintain that a measure of the pressor action of ergot enables us to estimate the value of the drug as an oxytoxic.

In an earlier paper, Wood and Hofer (21) pointed out that the use of ergot need not be limited to obstetrical practice. It is certainly true that a measure of the pressor activity of the drug upon lower animals would probably be a measure of the value of ergot as a circulatory stimulant for man, but it would seem that, in the light of both recent and early research, the objection to the use of an observation on the vasomotor action of ergot as a criterion of its oxytoxic power is decidedly a valid one.

Dale (4) has found that there is no constant relation between blood pressure and uterine effects when the drug was administered to a monkey. "In the cat, too, there is no distinct relation between the two sets of effects; some cats in which the blood pressure effect was comparatively small, showed marked uterine effects and vice versa."

In examining a number of commercial preparations of ergot, Goodall (9) also found that the absence of pressor activity did not justify one in assuming absence of power to affect the uterus. He concludes: "Whereas 42 per cent. of commercial samples caused contraction of the uterus without effecting a rise of blood pressure, the action on the uterus might be regarded as a more satisfactory test by the manufacturer."

Finally Edmunds and Hale, (7) after making a very careful comparison, have come to the conclusion that there is no essential parallelism between the two actions.

While Goodall's work is open to adverse criticism, the results secured by such men as Edmunds, Hale, and Dale must be considered as reliable and it would seem that some investigators have acted rather hastily in establishing arbitrary standards and making wholesale condemnations of commercial preparations without reporting a single experiment in support of their statements as to the parallelism between blood pressure and uterine effects.

The recent work of the English investigators offers an explanation as to why ergot may cause uterine contractions and yet fail to show pressor activity. Ergotoxine, para-hydroxyphenylethylamine, and B-isoamylamine excite the uterus to contractions and bring about an elevation of the blood pressure. B-iminazolylethylamine, however, is capable of causing intense uterine tetany, and, under proper conditions, a marked *fall* of systemic blood pressure.

While not essential, it is certainly advantageous to observe on lower animals the therapeutic action of the drug to be standardized. It seems most rational to attempt the standardization of the cardiac tonics by noting their effects upon the heart of the experimental animals; and, likewise, if the effect of ergot upon the uterus of a cat or some other suitable animal could be satisfactorily recorded, it would probably furnish a rational means for standardizing this drug. Numerous investigators have studied the action of ergot upon the uterus in various functional conditions, but, at present only two uterine methods are employed commercially.

Kehrer (13, 14) believes that the isolated uterus of a virgin cat offers an ideal object on which to standardize ergot. His method is simple in technic, but it would be extremely difficult to secure suitable animals in sufficient numbers for commercial standardization. Moreover, the light which recent chemical and physiological work has thrown upon the ergot question, enables us to see grave objections to Kehrer's method. In the first place, the important alkaloid, ergotoxine, in a pure state is insoluble in water, and Kehrer's test is, of necessity applicable only to the water soluble constituents. Then, of the amines present, two have an action similar to adrenaline in causing inhibition of the non-pregnant uterus. Edmunds and Hale (7) have found that there are serious practical disadvantages in the use of the excised uterus, the chief one arising from an increasing irritability of the organ, so that the employment of a solution of ergot which was too weak to have any effect at the begining of the experiment later on would cause distinct contractions.

Edmunds (8) was the first to make practical application of the method in which the movements of the uterus in situ are recorded. This method is obviously not simple and introduces the same theoretical disadvantages which were mentioned in discussing the isolated uterus method. Edmunds and Hale (7) find, however, that quite accurate results can be secured by perseverance and are inclined to consider this the most reliable method employed; an opinion in which Cushny (7) apparently concurs.

Finally, there is the much used and much abused cock's-comb method. As far back as 1824, Lorinser (14) noted the production of cyanosis in the comb of cocks to which ergot had been administered, and Kobert, Grunfeld, (10) and others made use of this phenomenon in studying ergot. Houghton, (11) however, was the first to make a practical application of it in attempting to standardize commercial preparations of the drug.

The cock's-comb method has, apparently, been employed by two classes of men in recent years. First, those who, believing implicitly in the efficiency of it as a method of standardization, have used it blindly without carefully investigating its worth. Second, a number of investigators who seemed to have paid too little attention to the attempt to secure uniform conditions, a factor of such great importance in any method of physiological assay.

Wood and Hofer (20) are inclined to consider the cock's-comb test of no value. They point out that there is "a wide biological gap between man and the chicken and the fact that the effect studied is a toxic one." Later, they say that they have made fifty experiments upon the rooster with such unsatisfactory results as to convince them that this method is too inaccurate to be of utility.

Cronyn and Henderson (3) concur in this opinion. They do not adduce any experimental evidence of their own in support of this contention, except reports of tests by them of preparations "of the one large pharmaceutical house whose preparations are supposed to be standardized by the same method."

The objection that "a wide biological gap exists between man and chicken" can have force only with those who fail to grasp the underlying principle of standardization. We wish to measure the oxytoxic principle in ergot, and it does not matter in what way it is measured if the measure be accurate. It certainly would seem that "a wide biological gap" exists between man on the one hand and the analytical balance on the other, yet this is not considered a sound reason for discarding methods of chemical assay.

Nor does it seem to us that the fact that the action upon the cock's comb is a toxic one proves the method defective. Lethal dose methods are sometimes accurate, and moreover, it is not so evident that the cyanosis of the comb is any more a "toxic" effect than an elevation of blood pressure 50 millimeters or stimulation of the uterus to marked contractions.

As Edmunds and Hale (7) have pointed out, Cronyn and Henderson are in error concerning Dale's statements as to variation in individual fowls of the same variety, since Dale (5) evidently used no one variety exclusively. The fact that preparations standardized by the cock's comb method seem inert when tested by Cronyn and Henderson is obviously no reason for criticizing the method, because there is apparently no way of knowing how old these preparations were when tested the second time.

In Wood and Hofer's fifty experiments, no mention is made of the number of fowls used; of the age, breed, or method of administration. It would certainly seem advisable for them to present full data before recommending the elimination of a method which has so much in its favor.

It remained for Edmunds and Hale (7) to take the first important step toward a solution of the question as to the best method available for commercial standardization of ergot. In their paper already quoted from, a careful comparison of various methods was made, and a striking similarity was found to exist between the results of tests upon the cat's uterus in situ and of tests upon fowls.

As would be expected, varying results have been secured when the question of deterioration of ergot was investigated by different methods. It is very surprising, however, to find that when presumably the same method was used by different men, variations fully as great appear in the results.

Grünfeld (10) was, so far as we can learn, the first to report systematic observations bearing upon the keeping qualities of ergot. His tests were made upon fowls and pigs, the drug being given per os. As the result of his experiments, he concluded that ergot, either powdered or in a natural state rapidly lost its activity and was practically worthless six months after the harvest. He also draws attention to the fact that the outbreaks of "ergotism" usually occur in the summer and autumn, when the ergot is fresh.

There are several features of Grünfeld's work, however, which allow opportunities for error. In the first place, his method of administration introduces the question of absorption from the gastro-intestinal canal. Secondly, he did not secure a stock of ergot and age it himself, making tests at various intervals, *but secured small lots as he needed them for testing,* assuming that their activity was the same throughout originally. Then, he disregarded the fact that seasonal variation may play an important part in the susceptibility of animals to poisons. Thus, Hunt (12) has shown that guinea pigs and mice vary in their resistance to acetonitrile according to the season of the year, and Südmersen and Glenny (19) find that the susceptibility of guinea pigs to poisoning by diphtheria toxin shows a similar variation. In our own laboratory, we have found a seasonal variation in guinea pigs and frogs when the heart tonics are used as toxic agents. While fowls may not show this seasonal variation, still the suspicion exists. Finally, he seemed to consider the death of the fowl as the end point, often disregarding temporary bluing of the comb.

Using practically the same method, Meulenhoff (15) reached very different conclusions. He believes that ergot kept under suitable conditions retains a considerable amount of activity for as long as five years.

Kehrer, (14) using the isolated uterus method, reaches conclusions approximating Grünfeld's. He states: "From this comparative investigation, it is evident that ergot, as preserved by the apothecary, in one year detereriorates 7-8 times; in two years, about 15 times."

Wood and Hofer (20) also find that ergot rapidly loses strength. By observations upon the blood pressure and by determination of the "sphacelotoxin content" these authors conclude:

"8. A fluid extract of ergot exposed to the air deteriorates extremely rapidly."

"9. The deteriorations of fluidextract of ergot may be much retarded by protecting it against contact with the air, but under the most favorable conditions there is a loss of strength approximating 10 per cent. a month."

If ergot loses in strength as rapidly as some of these authors believe, it is obvious that little can be expected of commercial preparations of the drug. When it is realized that Russian ergot does not reach the American manufacturers usually under six months after the harvest, and it is quite possible that it is mixed with ergot of the preceding year's harvest, it is evident that Grünfeld's statements being accepted would mean that ergot should be eliminated from the Pharmacopœia.

This state of affairs has existed for a number of years, and yet there are obstetricians who believe that satisfactory results follow the clinical use of ergot. Sharp (16) obtained a "liquid extract" of ergot which he kept under ordinary conditions for twelve months, using it, as the occasion offered, on patients in labor or who had just completed labor and in whom there was a "loss of uterine tone." He concluded that this liquid extract was apparently as active at the expiration as it was at the beginning of the year.

Wood and Hofer (20) and Crawford (2) apparently attach little value to the results of clinical observation in studying the question of the deterioration of ergot. It is certainly true that it is the tendency of some clinicians to draw conclusions from their experience in very limited numbers of cases, in which they have failed to consider adventitious circumstances. Such may have been true with Sharp, for he does not, unfortunately, give full enough details in regard to his study.

Bischofberger, (1) however, seems to have carried out his experiments with such care and thoroughness as to leave small room for questioning the results that he reports. In 1896, he tested lots of ergot, fresh, one year old, and two years old respectively, by administering the drug in powdered form to thirty patients. These patients had all been confined a short time previously and their abdominal walls were lax enough to permit of easy palpation of the uterus. In a number of instances, different lots of drug were tried upon the same patient, so that a comparison of activity could be made. He concludes: "If we take the mean of all experiments * * * it results that the activity of ergot of 1895 and 1896 is almost identical while that of 1894 * * * shows a plain, if only moderate decrease in activity." In comparing, more specifically, the results of experiments dealing only with fresh (1896) ergot and that one year old, he was able to see in the year-old specimen slightly less activity than in the fresh specimen.

As was said, Bischofberger's results seem entitled to acceptance, but, like Grünfeld he makes the mistake of assuming the same initial activity for his three lots of ergot. It is quite possible that his older ergot may have been considerably stronger when fresh than was the ergot of 1896, so his inferences as to the rate of deterioration are not justified. One point, however, seems established: namely, that ergot two years old has a decided influence upon the uterus of a woman when the drug is administered per os.

To study properly the question of the deterioration of ergot, it is necessary to secure a lot of the drug and determine its strength in reference to a definite standard, and then test it at stated intervals thereafter. Edmunds and Hale (7) seem to have shown that the cock's-comb method, carried out with proper precautions, is a satisfactory test method, and they suggest ergotoxine, a definite chemical compound, as a standard. An investigation of this nature is now being carried out in our laboratory, but it seemed to us that information of value could be obtained by examining fresh and old preparations of ergot with a view to determining their relative activity. Unfortunately, in our earlier work of the routine testing of ergot, we simply determined that the preparations tested were sufficiently active to cause bluing of the comb when injected intramuscularly in the dose of 1 cc. per kilo fowl, so the possibility remains that the older preparations were originally more active than the recent ones. We can not believe that this will be the case throughout the large number of samples tested, but would rather think that the more recent samples possess greater activity than those made several years ago, owing to the greater care in manufacture.

The samples were, at about the time when the preparation was made, placed in small one ounce, amber bottles. Ordinary cork stoppers were used, the samples were stored on shelves in a well lighted room and subjected to wide variations in temperature; in fact, they were kept under conditions similar to those in many retail pharmacies, except that for practically all of the samples the stoppers had not been removed during the time they remained in storage. In the few instances where the stoppers had been removed it was for the withdrawal of a small portion of the liquid for examination and occurred only once or twice in any instance.

Blood pressure experiments were carried out on dogs, using, in most cases, morphine anæsthesia, supplanted by ether for operation—the method proposed by Wood and Hofer.(20) The morphine was given subcutaneously one-half to one hour before the operation was begun. At the end of this period the animal was etherized, the common carotid artery was attached to a mercury manometer, and last the trachea was connected with the artificial respiration apparatus. After allowing the animal about ten minutes to recover from the ether, and when a constant blood pressure record had been secured, the injection was made. Artificial respiration was used in all experiments except one.

The systolic pressure (as recorded by the ordinary mercury manometer) was measured. Measurements were made in millimeters, and fractions of millimeters were dropped.

Table A gives the actual elevations of blood pressure at the beginning of the experiments, and for the several periods following. Table B gives the changes in pressure after the injection of the drug from that at the beginning of the experiments.

(+ = above, — = below.)

## TABLE A.

Blood Pressure Records on Dogs.

| Date of test. | No. of preparation. | Date when made. | Sex of dog. | Wt. of dog in Kgms. | Anaesthetic and amount given per Kgm. of body weight. Stated in fraction of gram. | No. of injection. | Dose per Kgm. stated in fractions of a c.c. | Blood pressure when inj. was made | Initial pressure (½-3 min. after inj ) | Pressure 5 min. after injection. | 10 min. after. | 15 min. after. | 20 min. after. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5-29-11 | 421352 | 5-29-11 | M | 6.6 | Morph. S. 0.008 | 1 | 0.10 | 136 | 170 | 169 | 172 | 164 | 161 |
| | | | | | | 2 | 0.15 | 160 | 178 | 168 | 158 | 150 | |
| 3-29-11 | 415330 | 3-26-11 | M | 17.0 | Morph. S. 0.006 | 1 | 0.08 | 132 | 159 | 157 | 151 | 141 | 151 |
| | | | | | | 2 | 0.16 | 150 | 164 | 173 | 172 | 174 | 180 |
| 2- 8-11 | 411173 | 2- 8-11 | M | 9.0 | Morph. S. 0.005 | 1 | 0.08 | 145 | 166 | 171 | 160 | | |
| | | | | | | 2 | 0.15 | 160 | 175 | 168 | | | |
| 2- 7-11 | 409578 | 2- 7-11 | M | 7.2 | Morph. S. 0.009 | 1 | 0 08 | 140 | 173 | 170 | 167 | | |
| | | | | | | 2 | 0.15 | 166 | 204 | 164 | | | |
| 12-21-10 | 406559 | 12-21-10 | M | 18.0 | Morph. S.<br>Atrop. S. | 1 | 0.14 | 163 | 234 | 226 | 215 | 200 | 190 |
| 12-18-10 | 406556 | 12-15-10 | M | 8.8 | Morph. S. 0.010<br>Atrop. S. 0.00014 | 1 | 0.05 | 114 | 218 | 214 | 187 | 187 | 166 |
| 11-18-10 | 401649 | 11-18-10 | F | 10.0 | Morph. S. 0.0064<br>Atrop. S. 0.00017 | 1 | 0.08 | 124 | 174 | 174 | 180 | 160 | 148 |
| 10-28-10 | 401646 | 10-28-10 | M | 13.6 | Morph. S.<br>Atrop. S. | 1 | 0.10 | 110 | 210 | 194 | 203 | 150 | 129 |
| 6-28-10 | 386647 | 6-28-10 | | 12.0 | Morph. S.<br>Acetoform<br>(No ether.) | 1 | 0.08 | 70 | 132 | 118 | 152 | 152 | 148 |
| 5-29-11 | 320659 | 9- 8-08 | M | 7.6 | Morph. S. 0.008<br>Acetoform .025 | 1 | 0.10 | 128 | 176 | 191 | 189 | 172 | 160 |
| 5-14-11 | 316825 | 8-24-08 | M | 6.3 | Morph. S. 0 006 | 1 | 0.10 | 159 | 173 | 179 | 188 | 200 | 200 |
| 4- 3-11 | 310299 | 6- 7-08 | M | 18.6 | Morph. S. 0.007 | 1 | 0.08 | 124 | 151 | 120 | 129 | 141 | 140 |
| | | | | | | 2 | 0.15 | 140 | 153 | 155 | 150 | 147 | 149 |
| 3-27-11 | 306389 | 5- 3-08 | F | 9.0 | Morph. S. 0.007 | 1 | 0.08 | 134 | 167 | 165 | 146 | 139 | 138 |
| | | | | | | 2 | 0.15 | 137 | 177 | 161 | 158 | 146 | 142 |
| 3-27-11 | 306389 | 5- 3-08 | M | 11.8 | Morph. S. 0.008 | 1 | 0.08 | 132 | 165 | 213 | Clot | | 152 |
| 3-23-11 | 277009 | 4-29-07 | F | 5.7 | Morph. S. | 1 | 0.08 | 95 | 135 | 115 | 122 | 132 | 157 |
| | | | | | | 2 | 0.15 | 137 | 160 | 137 | 146 | 122 | 120 |
| 3-23-11 | 277008 | 4-29-07 | F | 12.2 | Morph. S. 0.008 | 1 | 0.08 | 126 | Clot | 163 | 168 | 167 | 160 |
| 3-22-11 | 277007 | 4-23-07 | M | 15.4 | Morph. S. 0.007 | 1 | 0.08 | 174 | 201 | 138 | 207 | 216 | 148 |
| 6-24-11 | 272572 | 3-12-07 | M | 15.8 | Morph. S. 0.0037 | 1 | 0.10 | 118 | 149 | 128 | 111 | 106 | 102 |
| | | | | | Curare 0.0026 | 2 | 0.15 | 101 | 164 | 159 | 151 | 141 | 132 |
| 6-23-11 | 272572 | 3-12-07 | F | 18.4 | Morph. S 0.006 | 1 | 0.10 | 148 | 240 | 232 | 268 | 193 | 187 |
| | | | | | Atrop. S. 0.0001 | 2 | 0.15 | 170 | 242 | 222 | 199 | 188 | 184 |
| | | | | | Curare 0 0015 | | | | | | | | |
| 5- 5-11 | 261770 | 2-26-07 | M | 22.0 | Morph. S. 0.150 | 1 | 0.10 | 90 | 127 | 110 | 112 | 110 | 110 |
| | | | | | | 2 | 0.15 | 107 | 116 | 116 | 111 | 110 | 111 |
| 6-23-11 | 261766 | 12-22-06 | M | 20.0 | Morph. S. 0.006 | 1 | 0.10 | 142 | 160 | 167 | 165 | 157 | 153 |
| | | | | | Curare 0.0015 | 2 | 0.15 | 154 | 182 | 192 | 187 | 177 | |
| 6- 2-11 | 251766 | 12-22-06 | F | 7.1 | Morph. S. 0.0021* | 1 | 0.10 | 146 | 178 | 178 | 171 | 166 | 167 |
| | | | | | | 2 | 0.15 | 169 | 188 | 193 | 181 | 177 | 176 |
| 4- 4-11 | 255660 | 8-28-06 | M | 9.0 | Morph. S. 0.007 | 1 | 0.08 | 146 | 166 | 152 | 154 | 146 | 140 |
| | | | | | | 2 | 0.15 | 140 | 176 | 150 | 137 | | |
| 4-12-11 | 254053 | 8-16-06 | M | 19.5 | Morph. S. 0.010 | 1 | 0.10 | 122 | 181 | 170 | 140 | 136 | 130 |
| 4- 5-11 | 251604 | 8- 3-06 | M | 8.6 | Morph. S. 0.006 | 1 | 0.10 | 92 | 118 | 119 | 119 | 118 | 122 |
| | | | | | | 2 | 0.15 | 124 | 135 | 135 | 141 | 140 | 140 |
| 4- 5-11 | 251694 | 8- 3-06 | F | 4.3 | Morph. S. 0.0035 | 1 | 0 10 | 112 | 132 | 106 | 113 | 114 | 109 |
| 4- 5-11 | 251603 | 8-28-06 | M | 7.2 | Morph. S. 0.006 | 1 | 0.10 | 150 | 177 | 149 | 160 | 169 | 169 |

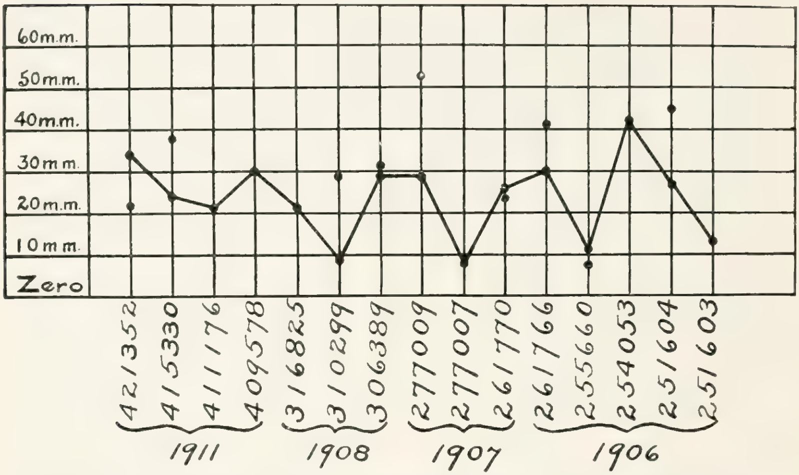
* Natural respiration.

TABLE B.

| No. of preparation. | Age. (Fractions of months were dropped.) | Anaesthetic. | No. of injection. | Initial change. | Change at end of 5 min. | 10 min. | 15 min. | 20 min. | Average change of initial, 5 and 10 min. periods. |
|---|---|---|---|---|---|---|---|---|---|
| 421352 | Fresh | Morph. S. | 1 | +34 | +33 | +36 | +28 | +25 | +34 |
| | | | 2 | +42 | +32 | +22 | +14 | | +22 |
| 415330 | Fresh | Morph. S. | 1 | +27 | +25 | +19 | +9 | +19 | +24 |
| | | | 2 | +34 | +41 | +40 | +42 | +48 | +38 |
| 411173 | Fresh | Morph. S. | | +21 | +26 | +15 | | | +21 |
| 409578 | Fresh | Morph. S. | | +33 | +30 | +27 | | | +30 |
| 406559 | Fresh | Morph. S. | | +71 | +63 | +52 | +37 | +27 | +62 |
| 406556 | Fresh | Atrop. S.<br>Morph. S. | | +104 | +100 | +73 | +73 | +52 | +92 |
| 401649 | Fresh | Atrop. S.<br>Morph. S. | | +50 | +50 | +56 | +36 | +24 | +52 |
| 401646 | Fresh | Atrop. S.<br>Morph. S. | | +100 | +84 | +93 | +40 | +19 | +92 |
| 386647 | Fresh | Atrop. S.<br>Morph. S. | | +62 | +48 | +82 | +82 | +78 | +64 |
| 320659 | 32 mo. | Acetoform<br>(No ether)<br>Morph. S. | | +48 | +63 | +61 | +44 | +32 | +57 |
| 316825 | 32 mo. | Acetoform<br>Morph. S. | | +14 | +20 | +29 | +41 | +41 | +21 |
| 310299 | 33 mo. | Morph. S. | 1 | +27 | —4 | +5 | +17 | +16 | +9 |
| | | | 2 | +29 | +31 | +26 | +23 | +25 | +29 |
| 306389 | 34 mo. | Morph. S. | 1 | +33 | +31 | +12 | +15 | +4 | +29 |
| | | | 2 | +43 | +27 | +24 | +12 | +8 | +31 |
| 306389 | 34 mo. | Morph. S. | | +33 | +81 | Clot | Clot | +20 | |
| 277009 | 46 mo. | Morph. S. | 1 | +40 | +20 | +27 | +37 | +42 | +29 |
| | | | 2 | +65 | +42 | +51 | +27 | +25 | +53 |
| 277008 | 46 mo. | Morph. S. | | Clot | +37 | +42 | +41 | +34 | |
| 277007 | 47 mo. | Morph. S. | | +27 | —36 | +33 | +42 | —26 | +8 |
| 272572 | 51 mo. | Morph S. | | +31 | +10 | —7 | —12 | —16 | +11 |
| | | Curare | | +46 | +41 | +33 | +23 | +14 | +40 |
| 272572 | 51 mo. | Morph. S. | 1 | +92 | +84 | +120 | +45 | +39 | +99 |
| | | Atrop. S. | 2 | +94 | +74 | +51 | +40 | +56 | +73 |
| 261770 | 50 mo. | Curare<br>Morph. S. | 1 | +37 | +20 | +22 | +20 | +20 | +26 |
| | | | 2 | +26 | +26 | +21 | +20 | +21 | +24 |
| 261766 | 54 mo. | Morph. S. | 1 | +18 | +25 | +23 | +15 | +11 | +22 |
| | | Curare | 2 | +40 | +50 | +45 | +35 | | +45 |
| 261766 | 53 mo. | Morph. S.* | 1 | +32 | +32 | +25 | +20 | +21 | +30 |
| | | | 2 | +42 | +47 | +35 | +31 | +30 | +41 |
| 255660 | 55 mo. | Morph. S. | 1 | +20 | +6 | +8 | 0 | —6 | +11 |
| | | | 2 | +30 | +4 | —9 | | | +8 |
| 254053 | 55 mo. | Morph. S. | | +57 | +48 | +18 | +14 | +8 | +42 |
| 251604 | 56 mo. | Morph. S. | 1 | +26 | +27 | +27 | +26 | +30 | +27 |
| | | | 2 | +43 | +43 | +49 | +48 | +48 | +45 |
| 251604 | 56 mo. | Morph. S. | | +20 | 6 | +1 | +2 | —3 | +5 |
| 251603 | 55 mo. | Morph. S. | | +27 | —1 | +10 | +19 | +19 | +12 |

* Natural respiration.

The average rise for the first three periods, in those experiments where morphine alone was used (with ether for operation), may be seen in plotted curve No. 1. The dots connected by lines represent pressures obtained from first injections. The seperate dots



Curve = Elevations in blood pressure of dogs, caused by the intravenous injection of fluidextracts of ergot, — morphine — ether anaesthesia.

Vertical Figures = Serial numbers of the fluidextracts.

Dates = When preparations were made

CURVE No. 1

represent pressures obtained from second injections (where second injections were made), and show the increase or decrease in pressure from that following the first injection. The vertical figures are the serial numbers of the fluidextracts. The dates at the bottom show when the preparations were made.

Five blood pressure experiments were carried out on spinal preparations, prepared according to the directions of Sherrington.(17) Data and results may be seen in Table C.

TABLE C.
Blood Pressure Records on Spinal Preparations.

| Date of test. | No. of preparation. | When made. | Animal. | Wt. in Kgms. | No. of injection. | Dose of drug per Kgm. in fraction of a c.c. | Blood pressure at beginning of inj. | Blood pressure ½-3 min. after injection. | 5 min. after. | 10 min. after. | 15 min. after. | 20 min. after. | Average rise of first three periods. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4- 5-11 | 251603 | 8-28-06 | Cat | 3.5 | 1 | 0.10 | 104 | 161 | 167 | 165 | 152 | 134 | +60 |
| 3-25-11 | 277007 | 4-23-07 | Cat | 2.6 | 1 | 0.08 | 130 | 136 | Clot | | 141 | 132 | |
| | | | | | 2 | 0.16 | 132 | 162 | 168 | | | | |
| 3-18-11 | 306389 | 5- 3-08 | Cat | 2.9 | 1 | 0.15 | 120 | 159 | 138 | 122 | 113 | | +20 |
| | | | | | 2 | 0.15 | 104 | 152 | 129 | 100 | | | |
| 5-14-11 | 316825 | 8-24-08 | Cat | 2.6 | 1 | 0.10 | 100 | 118 | 110 | | | | |
| | | | | | 2 | 0.10 | 96 | 139 | 125 | 88 | | | +21 |
| | | | | | 3 | 0.10 | 87 | 114 | 86 | | | | |
| 3-10-11 | 411176 | 2- 8-11 | Dog | 5.6 | 1 | 0.08 | 138 | 153 | 158 | 151 | 158 | | +16 |

The results secured by the blood-pressure experiments are surprising in view of the experience of Wood and Hofer. As may be seen from the table and from the chart, no definite information could be obtained by this method, for while some of the old samples failed to have the desired pressor activity, others appeared to be very active in this respect. In most of the experiments we followed the procedure of Wood and Hofer and are at a loss to account for the difference in the results.

Cock's-comb tests were carried out on stock samples of fluidextract (previously described). White Leghorn fowls from 7-10 months old were used except in a few cases where otherwise mentioned. All doses were calculated per kgm. of body weight. The drug was injected into the breast muscles and the fowls were observed at intervals for one and a half hours.

Fowls having well developed combs, of a deep red color and thickly covered with papillæ, have seemed to be the most susceptible, and will, nearly all of them, show the characteristic bluing of the comb provided the drug has been given in sufficient quantity.

Occasionally, however, fowls are found which will not show a bluing of the comb even after receiving large doses of the drug. In such chickens a blanching of the comb usually results, although in others there may be almost no change in color. Fowls of this kind were excluded. Some of those used may have partaken of this tendency in a slight measure. Certainly, some seemed more resistant than others. In some, considerable blanching of the comb would precede the bluing, while in others the comb would gradually become darker until a distinct bluing was visible. Of the fowls used, we made no selection for any given tests, but simply injected them in the order they happened to be brought to the laboratory by the assistant.

TABLE D.

Tests on Stock Samples of F. E. Ergot by the Cock's Comb Method, Using White Leghorns
Dose per Kgm. of body weight=0.75 c.c.

| Date of test. | No. of preparation. | When made. | No. of fowl. | Wt. in Kgms. | Result. |
|---|---|---|---|---|---|
| 11-14-11 | 434060 | 11-14-11 | 203 | 1.676 | Marked bluing + |
| 10-20-11 | 434057 | 10-20-11 | 194* | 1.849 | Faint bluing + |
| 10-10-11 | 429539 | 9-23-11 | 183 | 1.498 | No coloring O |
| 10-28-11 | 429539 | 9 23-11 | 191* | 1.965 | No coloring O |
| 11- 7-11 | 429539 | 9-23-11 | 195* | 1.735 | Faint bluing + |
| 11- 8 11 | 429539 | 9-23-11 | 186 | 1.511 | Faint bluing + |
| 11- 7-11 | 425456 | 9- 4-11 | 194* | 1.909 | Blanching O |
| 11- 8-11 | 425456 | 9- 4-11 | 187 | 1.250 | No coloring O |
| 10-10-11 | 425453 | 8- 8 11 | 172 | 1.224 | Marked bluing + |
| 10-10-11 | 421335 | 7-10-11 | 184 | 1.253 | Marked bluing + |
| 10 10-11 | 421352 | 6-11-11 | 181 | 1.231 | Faint bluing + |
| 10-11-11 | 415333 | 5-18-11 | 169 | 1.293 | Marked bluing + |
| 10-11-11 | 415330 | 4-27-11 | 171 | 1.207 | Marked bluing + |
| 10-11-11 | 411176 | 3-18-11 | 173 | 1.464 | Marked bluing + |
| 10-11-11 | 409575 | 2-10-11 | 168 | 1.105 | Very faint bluing + |
| 10-28-11 | 409575 | 2-10-11 | 192* | 1.912 | Very faint bluing + |
| 11- 7-11 | 409575 | 2-10 11 | 197* | 1.709 | Darkening O |
| 10-11-11 | 406559 | 1-19-11 | 182 | 1.412 | Faint bluing + |
| 10-13-11 | 401649 | 12-15-10 | 187 | 1.425 | Faint bluing + |
| 10-13-11 | 401646 | 11-22-10 | 186 | 1.420 | Marked bluing + |
| 10-13-11 | 397483 | 10-24-10 | 194 | 1.194 | Marked bluing + |
| 10-16-11 | 393872 | 9- 9-10 | 181 | 1.189 | No coloring O |
| 10-28-11 | 393872 | 9- 9-10 | 192* | 1.712 | Very faint bluing + |
| 10-16-11 | 393869 | 8-19-10 | 172 | 1.318 | Marked bluing + |
| 10-16-11 | 386647 | 7-17-10 | 184 | 1.146 | Marked bluing + |
| 10-16-11 | 382975 | 6- 4-10 | 183 | 1.438 | Faint bluing + |
| 10-17-11 | 382972 | 5- 6-10 | 186 | 1.579 | Marked bluing + |
| 10-17-11 | 380930 | 4-18-10 | 182 | 1.378 | Faint bluing + |
| 10-17-10 | 375495 | 3- 5-10 | 173 | 1.458 | Faint bluing + |
| 10-17-10 | 375491 | 2-19-10 | 171 | 1.151 | Very faint bluing + |
| 10-18-11 | 375488 | 1-28-09 | 187 | 1.400 | Very faint bluing + |
| 10-18-11 | 369507 | 12-10-09 | 190 | 1.218 | Very faint bluing + |
| 10-18-11 | 361919 | 11-14-09 | 189 | 1.339 | Very faint bluing + |
| 10-18-11 | 359337 | 9-12-09 | 188 | 1.231 | Very faint bluing + |
| 10-21-11 | 353756-7-8 | 8-21-09 | 185 | 1.106 | Darkening O |
| 10-30-11 | 353756-7-8 | 8-21-09 | 189 | 1.385 | No coloring O |
| 11-11-11 | 353756-7-8 | 8-21-09 | 198 | 1.565 | No coloring O |
| 10-21-11 | 353728 | 7-10-09 | 189 | 1.327 | Very faint bluing + |
| 10-21-11 | 351522 | 6-14-09 | 172 | 1.304 | Very faint bluing + |
| 10-21-11 | 347870 | 5-13-09 | 188 | 1.265 | Marked bluing + |
| 10-21-11 | 340670 | 4-10-09 | 173 | 1.341 | Darkening O |
| 10-30-11 | 340670 | 4-10-09 | 188 | 1.264 | Darkening O |
| 10-21-11 | 340668 | 3-22-09 | 190 | 1.234 | Very faint bluing + |
| 10-21-11 | 337592 | 2-27-09 | 187 | 1.380 | Darkening O |
| 10-21-11 | 337588 | 1-30-09 | 186 | 1.475 | Darkening O |
| 10-26-11 | 330033 | 12-23-08 | 183 | 1.480 | Darkening O |
| 10-26-11 | 323359 | 11-23-08 | 173 | 1.408 | Very faint bluing + |
| 10-26-11 | 323356 | 10-19-08 | 186 | 1.423 | Faint bluing + |
| 10-26-11 | 320661 | 9-20-08 | 185 | 1.100 | Darkening O |
| 10-26-11 | 316822 | 8- 3-08 | 190 | 1.269 | Darkening O |
| 10-26-11 | 316821 | 7-19-08 | 188 | 1.292 | Faint bluing + |
| 10-26-11 | 310300 | 6- 7-08 | 187 | 1.311 | Darkening O |
| 10-26-11 | 310298 | 5-15-08 | 189 | 1.380 | Faint bluing + |
| 10-28-11 | 306387 | 4-17-08 | 196° | 1.983 | Darkening O |
| 10-30-11 | 306387 | 4-17-08 | 186 | 1.372 | No coloring O |
| 10-28-11 | 303460 | 3-15-08 | 197° | 1.764 | No coloring O |
| 10-30-11 | 303460 | 3-15-08 | 185 | 1.065 | No coloring O |
| 10-30-11 | 300827 | 2- 4-08 | 183 | 1.461 | No coloring O |
| 10-30-11 | 300824 | 1- 4-08 | 187 | 1.249 | No coloring O |
| 10-30-11 | 294875 | 12- 6-07 | 169 | 1.085 | Very faint bluing + |
| 10-30-11 | 294874 | 11-23-07 | 172 | 1.306 | No coloring O |
| 10-30-11 | 291699 | 10-11-07 | 173 | 1.319 | No coloring O |
| 10-30-11 | 289208 | 9-13-07 | 190 | 1.200 | No coloring O |
| 10-30-11 | 286684 | 8-17-07 | 192* | 1.822 | No coloring O |
| 10 30-11 | 286682 | 7-14-07 | 191* | 1.804 | No coloring O |
| 10-30-11 | 280986 | 6-23-07 | 193* | 1.564 | No coloring O |
| 11- 4-11 | 277010 | 5-23-07 | 187 | 1.291 | Darkening O |
| 11- 4-11 | 277009 | 4-29-07 | 173 | 1.329 | No coloring O |
| 11- 4-11 | 272572 | 3-12-07 | 188 | 1.233 | Darkening O |
| 11- 4-11 | 261770 | 2-26-07 | 190 | 1.114 | Darkening O |
| 11- 4-11 | 251766 | 12-22-06 | 189 | 1.364 | Darkening O |
| 11- 4-11 | 255664 | 10- 8-06 | 172 | 1.295 | No coloring O |
| 11- 4-11 | 254053 | 8-16-06 | 183 | 1.479 | No coloring O |
| 11- 4 11 | 251604 | 8- 3-06 | 186 | 1.443 | No coloring O |

° Brown Leghorn at least one and one-half years old.
* Fowl at least one and one-half years old.

+=as above.
O=as above.

Tables D, E, and F show the results secured. In table D the samples were tested in a dose of 0.75 c.c.; in table E all samples failing to cause any bluing when administered in a dose of 0.75 c.c. were given in a dose of 1 c.c.; in table F those failing to cause bluing in 1 c.c. were administered in 1.5 c.c. per kgm.

The different results are designated in these tables as follows:

Marked bluing=Where larger part of comb was very distinctly blue.

Very faint bluing=Where the tips of points and back tip were very faintly though distinctly blue.

Faint bluing=A stage between the two preceding.

Darkening=Where comb seemed darkened but showed no distinct bluing.

Blanching=Where blanching occurred without any darkening or bluing.

TABLE E.

Tests on Stock Samples of F. E. Ergot by the Cock's Comb Method, Using White Leghorns. Dose per Kgm. of body weight=1 c.c.

| Date of test. | No. of Preparation | When made. | No. of fowl. | Wt. in Kgms. | Result. |
|---|---|---|---|---|---|
| 11-11-11 | 425456 | 9- 4-11 | 182 | 1.394 | Marked bluing + |
| 11-11-11 | 409575 | 2-10-11 | 173 | 1.298 | Marked bluing + |
| 11-11-11 | 353756-7-8 | 8-21-09 | 198 | 1.565 | No coloring O |
| 11-11-11 | 353756-7-8 | 8-21-09 | 186 | 1.455 | Very faint bluing + |
| 11- 7-11 | 340670 | 4-10-09 | 192* | 1.886 | Very faint bluing + |
| 11-11-11 | 340670 | 4-10-09 | 188 | 1.229 | Marked bluing + |
| 11-11-11 | 337592 | 2-27-09 | 187 | 1.218 | Darkening O |
| 11-11-11 | 337588 | 1-30-09 | 189 | 1.311 | Marked bluing + |
| 11-11-11 | 330033 | 12-23-08 | 185 | 1.089 | Darkening O |
| 11-11-11 | 320661 | 9-20-08 | 172 | 1.216 | Very faint bluing + |
| 11-11-11 | 316822 | 8- 3-08 | 190 | 1.117 | Marked bluing + |
| 11-11-11 | 310300 | 6- 7-08 | 181 | 1.298 | Very faint bluing + |
| 11-16-11 | 306387 | 4-17-08 | 182 | 1.423 | Darkening O |
| 11-16-11 | 303460 | 3-15-08 | 185 | 1.145 | Darkening O |
| 11-16-11 | 300827 | 2- 4-08 | 190 | 1.060 | Faint bluing + |
| 11-16-11 | 300824 | 1- 4-08 | 187 | 1.163 | Darkening O |
| 11-16-11 | 294874 | 11-23-07 | 188 | 1.173 | Faint bluing + |
| 11-16-11 | 291699 | 10-11-07 | 183 | 1.504 | Blanchihg O |
| 11-16-11 | 289208 | 9-13-07 | 181 | 1.276 | Darkening O |
| 11-16-11 | 286684 | 8-17-07 | 186 | 1.452 | Faint bluing + |
| 11-16-11 | 286682 | 7-14-07 | 189 | 1.262 | Marked bluing + |
| 11-16-11 | 280986 | 6-23-07 | 191* | 1.567 | Very faint bluing + |
| 11-16-11 | 277010 | 5-23-07 | 173 | 1.218 | Very faint bluing + |
| 11-16-11 | 277009 | 4-29-07 | 172 | 1.293 | Darkening O |
| 11-16-11 | 272572 | 3-12-07 | 192* | 1.687 | Blanching O |
| 11-18-11 | 261770 | 2-26-07 | 200 | 1.376 | Blanching O |
| 11-18-11 | 261766 | 12-26-06 | 199 | 1.793 | Very faint bluing + |
| 11-18-11 | 255664 | 10- 8-06 | 219 | 1.351 | Darkening O |
| 11-18-11 | 254053 | 8-16-06 | 220 | 1.390 | Darkening O |
| 11-18-11 | 251604 | 8- 3-06 | 198 | 1.715 | Darkening O |

* Fowl at least one and a half years old.

+=Bluing in any degree.

O=No noticable bluing.

TABLE F.

Tests on Stock Samples of F. E. Ergot by the Cock's Comb Method, Using White Leghorns. Dose per Kgm. of body weight=1.5 c.c.

| Date of test. | No. of Preparation | When made. | No. of fowl. | Wt. in Kgms. | Result. |
|---|---|---|---|---|---|
| 11-22-11 | 337592 | 2-27-09 | 188 | 1.210 | Very faint bluing + |
| 11-22-11 | 330033 | 12-23-08 | 187 | 1.206 | Darkening O |
| 11-22-11 | 306387 | 4-17-08 | 220 | 1.423 | Very faint bluing + |
| 11-22-11 | 303460 | 3-15-08 | 200 | 1.359 | Very faint bluing + |
| 11-22-11 | 300824 | 1- 4-08 | 202 | 1.522 | Very faint bluing + |
| 11-22-11 | 291699 | 10-11-07 | 173 | 1.260 | No coloring O |
| 11-22-11 | 289208 | 9-13-07 | 189 | 1.297 | Darkening O |
| 11-22-11 | 277009 | 4-29-07 | 186 | 1.532 | Very faint bluing + |
| 11-22-11 | 272572 | 3-12-07 | 182 | 1.430 | No Coloring O |
| 11-22-11 | 261770 | 2-26-07 | 190 | 1.089 | Darkening O |
| 11-22-11 | 255664 | 10- 8-06 | 181 | 1.298 | Very faint bluing + |
| 11-22-11 | 254053 | 8-16-06 | 172 | 1.366 | Very faint bluing + |
| 11-22-11 | 251604 | 8- 3-06 | 183 | 1.568 | No coloring O |

+=Bluing in any degree.
O=No noticable bluing.

Curve 2 was plotted from the results given in Tables D, E, and F and shows the response of fowls to the different doses of the fluid-extracts.

The gaps are due to the fact that preparations corresponding to those numbers did not cause bluing in a dose of 1.5 c.c. per kilo, that being the largest dose used in this series.

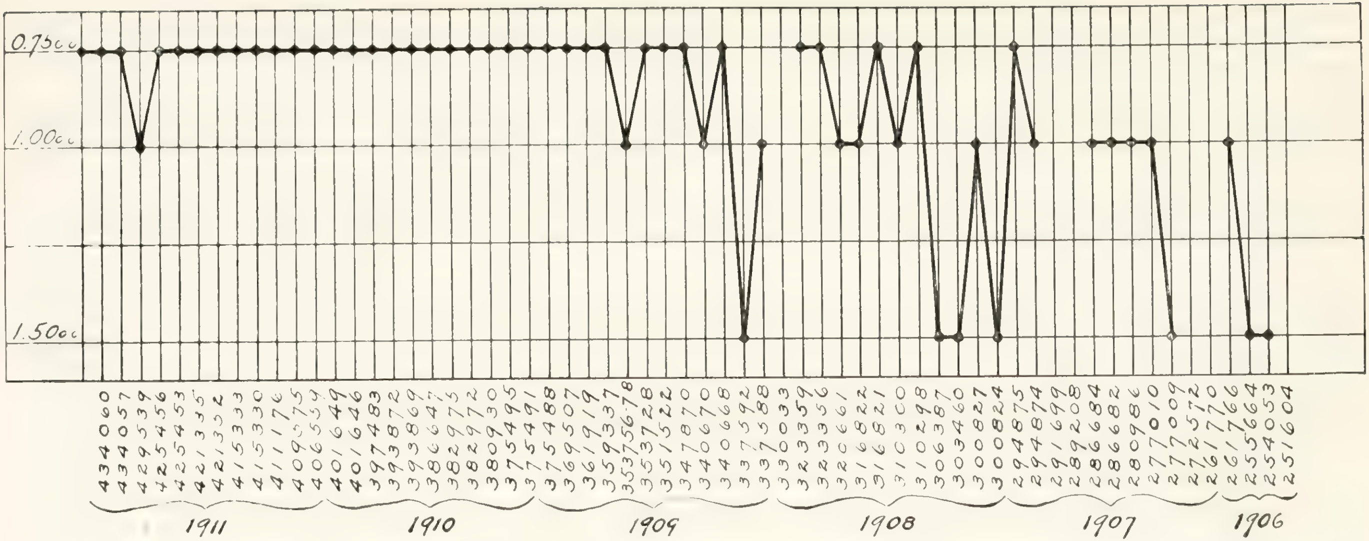
## TESTS OF MIXED SAMPLES MADE FROM STOCK SAMPLES BY THE COCK'S COMB METHOD, USING WHITE LEGHORNS.

Sixty of the stock lot samples previously tested individully were divided into five groups of twelve each. A mixed sample was made from each group. Since the individual samples were taken, one for each month, extending back over approximately sixty months, each mixed sample represented the lots made during a given year. The sample from the oldest group was labeled 1, the next 2, 3, 4, and 5 respectively. Data and results may be seen in table G, or plotted in curve 3.

From an examination of Table D, it is seen that, with a few exceptions, the samples back to April 10, 1909 (that is, two years and six months), seem to cause bluing of the comb in a dose of 0.75 c.c. per kgm. From that time back, of the 31 samples tested, only six had any visible effect upon the comb when glven in this dose.

From Table E, it may be seen that, with quite a number of exceptions, there is no distinct evidence of diminished strength until April 29, 1907 (sample No. 277009), or about four years and six months.

Table F does not give information of any value, because the



This curve shows the response by fowls to different doses of the individual samples of fluidextract of ergot Plotted from tables D E and F
Vertical numbers = Serial numbers of the fluidextracts Dates = Year when preparations were made

Curve No 2

TABLE G.

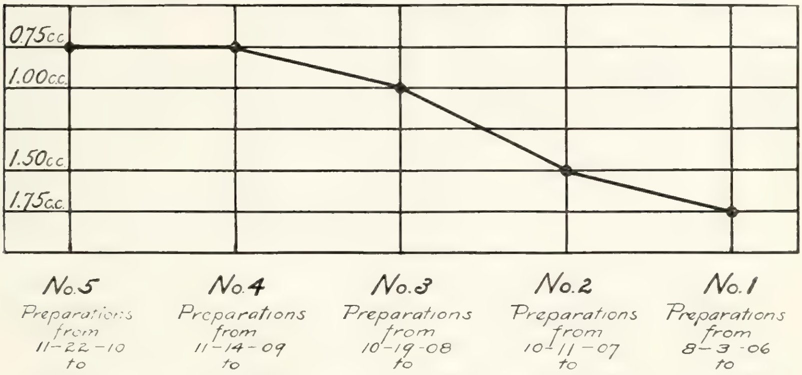
Tests on Stock Samples of F. E. Ergot by the Cock's Comb Method, Using White Leghorns Dose per Kgm. of body weight=0.75 c.c.

| Date of test. | Sample and age | No. of fowl. | Wt. in Kgms. | Dose per Kgm. | Result. |
|---|---|---|---|---|---|
| 11-14-11 | 1 Preparations from 8- 3-06 to 9-13-07 inclusive | 202 | 1.469 | 0.75 c.c. | No coloring O |
| 11-22-11 | | 198 | 1.704 | 0.75 c.c. | Blanching O |
| 11-18-11 | | 233 | 1.865 | 1.00 c.c. | Darkening O |
| 12- 5-11 | | 199 | 1.953 | 1.25 c.c. | Very faint bluing + |
| 12- 5-11 | | 224 | 1.368 | 1.25 c.c | Darkening O |
| 12- 9-11 | | 223 | 1.850 | 1.25 c.c. | Darkening O |
| 11-28-11 | | 226 | 1.586 | 1.5 c.c. | Faint bluing + |
| 12-12-11 | | 202 | 1.558 | 1.5 c.c. | Darkening O |
| 12-12-11 | | 225 | 1.793 | 1.5 c.c. | No coloring O |
| 12-21-11 | | 201 | 1.450 | 1.5 c.c. | Very faint bluing + |
| 12-21-11 | | 237 | 1.437 | 1.5 c.c. | No coloring O |
| 12-21-11 | | 220 | 1.475 | 1.63 c.c. | Very faint bluing + |
| 12-21-11 | | 187 | 1.392 | 1.63 c.c. | Very faint bluing + |
| 12-21-11 | | 226 | 1.500 | 1.63 c.c. | Darkening O |
| 12-15-11 | | 203 | 1.796 | 1.75 c.c. | Very faint bluing + |
| 12-21-11 | | 204 | 1.371 | 1.75 c.c. | Faint bluing + |
| 11-14-11 | 2 Preparations from 10-11-07 to 9-20-08 inclusive | 201 | 1.507 | 0.75 c.c. | Darkening O |
| 11-14-11 | | 226 | 1.726 | 0.75 c.c. | Darkening O |
| 11-18-11 | | 222 | 1.396 | 1.00 c.c. | Darkening O |
| 12- 6-11 | | 223 | 1.907 | 1.00 c.c. | Darkening O |
| 12- 6-11 | | 198 | 1.725 | 1.00 c.c. | Blanching O |
| 12- 9-11 | | 219 | 1.397 | 1.00 c.c. | No coloring O |
| 12-12-11 | | 204 | 1.478 | 1.25 c.c. | Very faint bluing + |
| 12-15-11 | | 202 | 1.418 | 1.25 c.c. | Faint bluing + |
| 12-15-11 | | 226 | 1.514 | 1.25 c.c. | Darkening O |
| 12-28-11 | | 222 | 1.398 | 1.50 c.c. | Faint bluing + |
| 12-12-11 | | 199 | 1.916 | 1.50 c.c. | Very faint bluing + |
| 12-12-11 | | 237 | 1.541 | 1.50 c.c. | Very faint bluing + |
| 12- 6-11 | 3 Preparations from 10-19-08 to 9-12-09 inclusive | 200 | 1.348 | 0.50 c.c. | Darkening O |
| 12- 6-11 | | 202 | 1.559 | 0.63 c.c. | Darkening O |
| 12- 9-11 | | 226 | 1.558 | 0.63 c.c. | No coloring O |
| 11-14-11 | | 200 | 1.418 | 0.75 c.c. | Very faint bluing + |
| 11-22-11 | | 202 | 1.721 | 0.75 c.c. | Faint bluing + |
| 12-12-11 | | 200 | 1.493 | 0.75 c.c. | Darkening O |
| 12-12-11 | | 226 | 1.550 | 0.75 c.c. | No coloring O |
| 12-15-11 | | 223 | 1.841 | 0.87 c.c. | No coloring O |
| 12-15-11 | | 224 | 1.384 | 0.87 c.c. | No coloring O |
| 12-21-11 | | 225 | 1.764 | 0.87 c.c. | Darkening O |
| 12-21-11 | | 223 | 1.878 | 0.87 c.c. | Darkening O |
| 12-21-11 | | 182 | 1.689 | 0.87 c.c. | Very faint bluing + |
| 11-18-11 | | 201 | 1.477 | 1.00 c.c. | Faint bluing + |
| 12-21-11 | | 219 | 1.327 | 1.00 c.c. | Very faint bluing + |
| 12-21-11 | | 224 | 1.419 | 1.00 c.c. | Very faint bluing + |
| 12- 6-11 | 4 Preparations from 11-14-09 to 10-24-10 inclusive | 226 | 1.576 | 0.5 c.c. | No coloring O |
| 12- 6-11 | | 225 | 1.806 | 0.5 c.c. | No coloring O |
| 12- 9-11 | | 224 | 1.355 | 0.63 c.c. | Darkening O |
| 15-15-11 | | 225 | 1.739 | 0.63 c.c. | Very faint bluing + |
| 12-15-11 | | 187 | 1.333 | 0.63 c.c. | Darkening O |
| 12-21-11 | | 229 | 1.193 | 0.63 c.c. | Very faint bluing + |
| 12-21-11 | | 199 | 1.908 | 0.63 c.c. | No coloring O |
| 12-21-11 | | 199 | 1.738 | 0.75 c.c. | Very faint bluing + |
| 11-22-11 | | 225 | 1.933 | 0.75 c.c. | Very faint bluing + |
| 11-18-11 | | 221 | 1.566 | 1.00 c.c. | Marked bluing + |
| 12- 6-11 | 5 Preparations from 11-22-10 to 10-20-11 inclusive | 203 | 1.775 | 0.5 c.c. | No coloring O |
| 12- 6-11 | | 219 | 1.387 | 0.5 c.c. | No coloring O |
| 12- 9-11 | | 225 | 1.744 | 0.63 c.c. | Darkening O |
| 12-15-11 | | 201 | 1.429 | 0.63 c.c. | No coloring O |
| 12-15-11 | | 219 | 1.367 | 0.63 c.c. | No coloring O |
| 12-21-11 | | 186 | 1.669 | 0.63 c.c. | Very faint bluing + |
| 12-21-11 | | 228 | 1.465 | 0.63 c.c. | Very faint bluing + |
| 11-14-11 | | 198 | 1.760 | 0.75 c.c. | Very faint bluing + |
| 11-22-11 | | 201 | 1.473 | 0.75 c.c. | Faint bluing + |
| 11-18-11 | | 203 | 1.647 | 1.00 c.c. | Marked bluing + |

sample five years old gave as much bluing in the dose of 1.5 c.c. as did the one only two years old.

The results given in Table G are, we believe, the ones of most value. By mixing together all of the samples for one year, variations of individual samples become less important and the resulting diminution in the number of preparations enables fairly accurate comparison of strength.

For mixture No. 1, we may put the effective dose at 1.75 c.c.; or may say that a mixture of preparations about four and one-half years old causes bluing of the cock's comb when given in that dose. Mixture No. 2 approximately three and one-half years old, produced about the same effect when given in a dose of 1.5 c.c. Mixture No. 3, about two and one-half years old, produces bluing in a dose of 1.00 c.c.; while mixture No. 4, about one and one-half years old, and mixture No. 5, about six months old, were effective in a dose of 0.75 c.c. The last two mixtures showed an almost identical degree of activity, so far as we could determine, and it is exceptional to find a fresh preparation that will cause bluing in a smaller dose than this. Edmunds and Hale* find that 1 c.c. of a fresh fluidextract of ergot per kgm. fowl should cause distinct bluing of the comb, and suggest that 5 mgms. of ergotoxine phosphate be considered equivalent to 2 c.c. of such a fluidextract. On our chickens, we have found that our effective dose of 0.75 c.c. is about equivalent to 1.87 mgms. of ergotoxine phosphate; or, in other words, samples of fluidextract of ergot two years old possess an activity approximately equal to this provisional standard.



This curve shows the amount of the different mixed samples of fluidextract of ergot required to produce bluing of the cock's comb. Curve plotted from table G.

CURVE No. 3

The mixture two and one-half years old had to be given in a dose 33 1-3 per cent. larger; the three and a half year old mixture had to be given in a dose 100 per cent. larger; while the four and one-half year old mixture was effective only in a dose about 133 per cent. larger than the mixtures made up of comparatively fresh preparations.

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* These authors do not state distinctly that the dose was per kgm., but from the context we assume this.

As we have said, inferences as to the exact rate of deterioration can be drawn from these experiments only on the assumption of an initial activity the same for all preparations. This is, of course, not justified, but it does seem to us that the large number of preparations examined affords ground for making approximations as to the keeping quality of the fluidextract of ergot. It would seem to us that, if the cock's-comb method is considered reliable, preparations of the fluidextract of ergot kept in well-stoppered, small vials under ordinary conditions will retain their activity practically unaltered for at least two or two and one-half years, but from this time on there is an appreciable deterioration which, at the end of four or five years, would necessitate the administration of more than double the does in order to secure the same effect as from a fresh preparation. It is probable that deterioration is taking place from the time of manufacture, but in the samples examined by us, this did not become evident under two and a half years.

It has been frequently suggested that commercial preparations of ergot should bear the date when tested, but it seems to us that such procedure would be of value only when information is possessed concerning the probable rate of deterioration. The experiments which we report are suggestive, but we hope that others will take up the investigation so that a definite conclusion can be reached.

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# EXPERIMENTS WITH THE CAT METHOD FOR TESTING DIGITALS AND ITS ALLIES.

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There are at present four American methods in use for the physiological testing of the digitalis series, namely: The twelve hour frog method, proposed by Houghton; the one-hour frog-heart method, proposed by Famulener and Lyons; the guinea pig method, proposed by Reed and Vanderleed; and the cat method, proposed by Hatcher and Brody.

In view of the large amount of work which is being carried out just now with these methods, with the hope that some one may be found sufficiently accurate and convenient to justify its insertion into the next Pharmacopœia, it seemed proper to report some results obtained with one of these—the cat method of Hatcher and Brody. They describe their method in the American Journal of Pharmacy for August, 1910, and state that it is an accurate one, and one that can be conveniently carried out by the retail pharmacist. The purpose of this study was to ascertain with what ease the method could be used, and what uniformity of results could be obtained. Since they have not given all the details of manipulation, I will describe the method as I used it, my endeavor being to carry it out in all respects just as they did.

Fully grown apparently healthy cats were selected. In general these were stray cats of the city, and represented all common breeds and mixtures. They were accurately weighed, and then anæsthetized in the following manner: The animal was placed in a small box, just large enough to accommodate the body, with a small circular notch at the top of one end of a size which would just admit the neck. A cat in the box with the neck in place, the sliding lid was forced shut and held by a peg. Thus the animal was unable to withdraw its head. The anæsthetic was then given from a small copper cone carrying on a transverse screen a pad of cotton or gauze. A few drops of chlorofrom were placed on the cotton at the start in order to hasten this operation. As soon as the animal was unconscious this pad of cotton was replaced by another upon which only ether was dropped. The cat was then tied on an animal board (somewhat resembling the Harvard) with back down, legs outstretched, and head securely fastened in a holder. This board supported on legs, was made so as to drain at a point near the lower (tail) end, under which a receiving vessel was placed. The animal in place, the femoral veins were dissected out and small glass cannulæ inserted. The solutions were contained in burettes, the ouabain in one and the digitalis body in the other, and were conveyed to the cannulæ by narrow catheter tubing. The injection extended, as near as could be arranged, over a period of ninety minutes.

## THE OUABAIN SOLUTION.

Merck's crystalline ouabain was used. The weighing was done on an accurate chemical balance, and a stock solution 1:10,000 was made in a one litre volumetric flask. For use, samples were drawn off with a pipette and diluted to the strength 1:100,000 in a narrow glass-stoppered 200 c.c. cylinder. All dilutions were made with recently prepared physiological salt solution. (0.75 per cent. NaCl.) The stock solution was kept in a cool dark cupboard, and in no case was used after two weeks old. The solution for use was made up as needed.

After running two preliminary experiments to become acquainted with the technic, a series of twenty-six experiments with ouabain was begun. The procedure and results were as follows:

The weight of the animal having been taken, the theoretical amount of solution required was calculated. Since the lethal dose of ouabain for cats, according to Dr. Hatcher, is .000,1 gramme per kilogramme of body weight, the theoretical amount of solution would be the number of cubic centimeters required, for any given animal, to supply .000,1 Gm. per Kgm. And since each cubic centimeter of a 1 :100,000 solution would contain .000,01 Gm., a 3.2 Kgm. cat, for example, would require 32 c.c. Ninety minutes being the period of injection, the proportionate part of the theoretical amount necessary to be run in each minute or two minutes, was calculated. The operator seated at the table, continued the anæsthesia by placing a small pad of gauze over the nose and supplying only sufficient ether to just keep the animal quiet. The burette having been filled and the time noted, the injection was begun, running in slowly every minute or two minutes the amount proportioned. The cat was carefully watched, particularly toward the end when the larger part of the theoretical amount had been injected. Death was usually preceded by very rapid respiration and decided convulsive movements, after which the respiration ceased to be regular and was prolonged for a few moments only by gasps. As soon as these symptoms of approaching death appeared, the injection was stopped. If after waiting a few minutes the animal did not die, the injection was continued very slowly and with great caution. When respiration had ceased to be regular, the number of cubic centimeters of solution used and the time were noted, and before the gasping had entirely ceased the heart was exposed. In the majority of cases, rhythmic contractions of the heart had ceased. Sometimes the heart was in feeble delirium, but usually the left ventricle was still and the other chambers were feebly contracting. Out of twenty-six experiments with ouabain, sixteen with strophanthus, and twenty-seven with digitalis, only seven hearts were found beating rhythmically, and in these the contractions were very feeble. Twelve hearts showed the left ventricle in quite complete systole. It should be remembered that regular respiration had ceased from one to three minutes previous to the exposure of these hearts. In one instance under ouabain, paraldehyde was used as the anæsthetic. (1.8 c.c. per Kgm. Merck's.) Immediately upon appearance of the gasping, without opening the thorax, artificial respiration was instituted. The heart seemed to improve, and continued to beat until at the end of ten minutes one cubic centimeter more of the solution was slowly injected when it stopped. With cat No. 26 artificial respiration was supplied all through the experiment, still the animal died within the ninety minutes, having received almost the exact theoretical amount. To accurately determine the effect of artificial respiration upon the lethal dose would of course require a large number of experiments, an interesting point, but one I have not been able to work out for lack of time and animals.

OUABAIN

| Date | Cat No. | Sex | Wt. in Kgms. | C.c. Sol. | Ouabain in Gms. per Kgm. | Time in. Min. |
|---|---|---|---|---|---|---|
| 12-16-10 | 1 | Male | 2.10 | 25.0 | .000,119 | 99 |
| 12-18-10 | 2 | Male | 4.70 | 32.0 | .000,068 | 70 |
| 12-21-10 | 3 | Male | 2.80 | 25.0 | .000,089 | 71 |
| 12-22-10 | 4* | Male | 2.00 | 15.0 | .000,075 | 75 |
| 12-24-10 | 5 | Female | 1.10 | 11.5 | .000,104 | 118 |
| 12-24-10 | 6 | Male | 1.80 | 16.8 | .000,093 | 87 |
| 1- 4-11 | 7 | Male | 1.70 | 16.0 | .000,094 | 93 |
| 1- 5-11 | 8 | Male | 2.70 | 16.0 | .000,060 | 61 |
| 1-30-11 | 9 | Female | 1.19 | 11.2 | .000,094 | 88 |
| 1-31-11 | 10 | Male | 2.11 | 22.1 | .000,105 | 94 |
| 2- 1-11 | 11 | Female | 1.77 | 17.4 | .000,098 | 95 |
| 2- 2-11 | 12 | Female | 2.33 | 19.6 | .000,084 | 82 |
| 2- 2-11 | 13 | Female | 0.97 | 13.0 | .000,134 | 104 |
| 2- 3-11 | 14 | Female | 2.13 | 27.0 | .000,126 | 106 |
| 2- 4-11 | 15 | Male | 3.42 | 46.0 | .000,134 | 106 |
| 2- 4-11 | 16 | Female | 2.43 | 26.5 | .000,109 | 97 |
| 2-10-11 | 17 | Male | 2.50 | 23.0 | .000,092 | 82 |
| 2-11-11 | 18 | Male | 3.27 | 32.7 | .000,100 | 91 |
| 2-13-11 | 19 | Male | 2.94 | 25.5 | .000,086 | 77 |
| 2-13-11 | 20 | Male | 1.81 | 17.5 | .000,096 | 86 |
| 2-14-11 | 21 | Male | 2.40 | 20.0 | .000,083 | 76 |
| 2-14-11 | 22 | Female | 1.87 | 16.0 | .000,085 | 83 |
| 2-15-11 | 23 | Male | 2.28 | 22.0 | .000,096 | 90 |
| 2-15-11 | 24 | Female | 2.25 | 21.0 | .000,093 | 87 |
| 2-15-11 | 25 | Female | 1.92 | 13.5 | .000,070 | 65 |
| 6-14-11 | 26 | Female | 2.38 | 24.0 | .000,100 | 87 |

* Received paraldehyde instead of ether.

Two samples of strophanthus seed (Kombe) were received for testing at this time. These were reduced to No. 60 powder. Ten gram samples were placed in 150 c.c. Erlenmeyer flasks, supplied with good, tightly fitting corks, and macerated with 40 c.c. of 75 per cent. alcohol for 72 hours with occasional agitation. The content of each flask was then poured into a small narrow percolator fitted at the neck with a tight pluget of cotton. The first portion of each percolate was returned and the percolation was then allowed to proceed at the rate of 10 drops per minute. Seventy-five per cent. alcohol was added from time to time until 200 c.c. of percolate were obtained, thus finishing a 5 per cent. tincture. For injection, 1:6,000 solutions were used. These were made in the same manner as described under ouabain. The results from eight experiments on each of these samples were as follows:

STROPHANTHUS SEED No. B-565.

Dilution 1:6,000 1 c.c. = .000,166 gm. drug.

| Date | Cat No. | Sex | Wt. in Kgms. | C.c. Sol. | Strophanthus in Gm. | Time |
|---|---|---|---|---|---|---|
| 2-16-11 | .... 1 | Male | 2.23 | 16.0 | .001,19 | 69 |
| 2-16-11 | .... 2 | Male | 2.65 | 22.0 | .001,37 | 100 |
| 2-16-11 | .... 3 | Female | 2.82 | 26.0 | .001,53 | 98 |
| 2-17-11 | .... 4 | Male | 2.29 | 22.9 | .001,66 | 107 |
| 2-17-11 | .... 5 | Male | 2.11 | 24.0 | .001,88 | 100 |
| 2-18-11 | .... 6 | Male | 2.98 | 30.0 | .001,66 | 95 |
| 2-18-11 | .... 7 | Male | 3.07 | 31.0 | .001,67 | 100 |
| 2-18-11 | .... 8 | Female | 2.04 | 18.0 | .001,46 | 66 |

STROPHANTHTS SEED No. B-566.

| Date | Cat No. | Sex | Wt. in Kgms. | C.c. Sol. | Strophanthus in Gm. | Time |
|---|---|---|---|---|---|---|
| 2-21-11 | .... 1 | Male | 2.45 | 26.4 | .001,78 | 102 |
| 2-21-11 | .... 2 | Male | 3.17 | 23.0 | .001,20 | 60 |
| 2-21-11 | .... 3 | Male | 1.29 | 11.1 | .001,42 | 71 |
| 2-22-11 | .... 4 | Male | 3.00 | 24.0 | .001,32 | 60 |
| 2-22-11 | .... 5 | Male | 3.17 | 25.5 | .001,33 | 87 |
| 2-22-11 | .... 6 | Female | 3.14 | 23.5 | .001,23 | 87 |
| 2-23-11 | .... 7 | Male | 3.82 | 29.0 | .001,24 | 95 |
| 2-23-11 | .... 8 | Male | 2.93 | 25.0 | .001,41 | 111 |

Hatcher and Brody have found after many experiments that if digitalis and the other members of the series are injected, like ouabain and strophanthus, until the animal dies, the results will usually be too high—necessitating a correction of about 20 per cent. They have therefore devised a modification of the method which gives results comparable in accuracy, they believe, to those obtained with crystalline ouabain itself. This modification is as follows:

A measured quantity of the digitalis solution (I understand about 50 per cent of the required amount) is injected during the first period of about ten minutes. After an interval of about twenty minutes the injection is continued, substituting ouabain solution for the digitalis, until the animal dies. The difference between the amount of ouabain actually used to complete the experiment, and the theoretical amount necessary to kill the animal in the absence of the digitalis body, represents the amount of ouabain to which the digitalis body used is equivalent. The amount of digitalis body equivalent to .000,1 Gm. or one "cat unit," is then calculated.

### EXAMPLE TO SHOW METHOD OF CALCULATION.

| | |
|---|---|
| Digitalis solution =1 : 100 | 1 c. c. =.010 Gm. |
| Ouabain solution =1 : 100,000 | 1 c. c. =.000,01 Gm. |

Cat weighing 3.21 Kgms. received { 30.2 c. c. digitalis sol. (.302 Gm. drug) or, .0940 Gm. drug per Kgm. body weight; 5.5 c. c. ouabain sol. (.000,055 Gm. ouabain) or, .000,017 Gm. ouabain per Kgm. of cat.

The difference between .000,017 Gm., the amount of ouabain (per Kgm.) actually used to complete the experiment, and .000,100 Gm., the theoretical amount, or one "cat unit," which would have been required in the absence of the digitalis body, is .000,083 Gm.

.094 Gm. of the digitalis is therefore equivalent to .000,083 Gm. ouabain, or .094 Gm. of the digitalis=83 per cent. of one "cat unit."

.113 Gm. of the digitalis would then be equivalent to one "cat unit."

### F. E. DIGITALIS.

No. 405467.

Digitalis dilution 1:100
Ouabain dilution 1:100,000

| Date | Cat No | Sex | Wt. in Kgms. | c. c. Dig. Sol. | c. c. Ouabain sol. | Equiv. in Gm. of 1 cat unit | Time in Min. |
|---|---|---|---|---|---|---|---|
| 2-27-11 | 1 | Female | 3.21 | 30.2 | 5.5 | .113,5 | 95 |
| 2-27-11 | 2 | Male | 2.88 | 25.0 | 3.0 | .096,8 | 61 |
| 2-27-11 | 3 | Male | 2.87 | 25.0 | 7.0 | .115,2 | 100 |

### F. E. DIGITALIS.

No. 416233.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5- 8-11 | 1 | Male | 2.33 | 14.0 | 5.2 | .077,3 | 66 |
| 5- 8-11 | 2 | Female | 2.18 | 12.0 | 3.6 | .065,8 | 65 |
| 5- 9-11 | 3 | Male | 2.07 | 11.0 | 4.6 | .068,2 | 80 |
| 5- 9-11 | 4 | Female | 1.83 | 10.0 | 6.7 | .086,1 | 73 |
| 5-10-11 | 5 | Male | 2.21 | 11.1 | 6.0 | .068,8 | 97 |
| 5-10-11 | 6 | Male | 2.40 | 14.0 | 8.0 | .087,4 | 82 |

### F. E. DIGITALIS.
### No. 335929.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5-11-11 | 1 | Female | 1.72 | 10.0 | 3.5 | .072,8 | 79 |
| 5-11-11 | 2 | Male | 3.18 | 15.0 | 21.0 | .138,7 | 114 |
| 5-11-11 | 3 | Female | 1.75 | 11.7 | 9.0 | .137,5 | 100 |
| 5-12-11 | 4 | Female | 1.89 | 10.0 | 10.0 | .112,3 | 90 |
| 5-13-11 | 5 | Female[1] | 2.15 | 10.0 | 15.0 | .153,4 | 155 |
| 5-15-11 | 6 | Female[1] | 2.55 | 12.0 | 25.5 | .522,7 | 114 |
| 5-15-11 | 7 | Female[1] | 1.82 | 12.0 | 12.0 | .193,5 | 80 |
| 5-16-11 | 8 | Male | 3.00 | 16.0 | 17.5 | .127,9 | 115 |
| 5-17-11 | 9 | Female[1] | 2.90 | 17.5 | 9.7 | .090,6 | 75 |
| 5-18-11 | 10 | Male | 2.46 | 16.0 | 7.0 | .090,8 | 75 |
| 5-18-11 | 11 | Female | 1.98 | 11.0 | 8.0 | .093,2 | 85 |

[1] In varying stages of lactation.

### TR. DIGITALIS NO. 2-B

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5-22-11 | 1 | Female | 2.21 | 13.0 | 3.5 | .073,2 | 61 |
| 5-23-11 | 2 | Male | 2.89 | 14.0 | 16.5 | .112,6 | 95 |
| 5-23-11 | 3 | Female[1] | 2.35 | 13.0 | 15.0 | .154,2 | 85 |
| 5-24-11 | 4 | Male | 2.83 | 17.0 | 3.0 | .067,1 | 111 |
| 5-24-11 | 5 | Female[2] | 2.46 | 15.0 | 14.0 | .141,4 | 106 |
| 5-24-11 | 6 | Male | 2.30 | 16.4 | 8.0 | .109,3 | 81 |
| 5-25-11 | 7 | Female | 3.00 | 16.0 | 7.5 | .071,1 | 73 |

[1] Lactating.

[2] Apparently in period immediately following lactation. Glands were still enlarged, but not functionating.

## ASSAYS ON FOREGOING PREPARATIONS BY OTHER METHODS.

### ONE-HOUR FROG-HEART METHOD. VARIETY RANA PIPIENS. TEMPETATURE 20° C.

### STROPHANTHUS SEED B-565.

| Weight of frog in Grammes | Dose per Gramme | Result |
|---|---|---|
| 36.5 | .000,006,0 | Stopped |
| 40.8 | .000,005,0 | ” |
| 15.1 | .000,005,0 | ” |
| 28.1 | .000,004,0 | ” |
| 39.6 | .000,004,0 | ” |
| 23.0 | .000,004,0 | ” |
| 43.8 | .000,003,5 | ” |
| 36.4 | .000,003,5 | ” |
| 48.7 | .000,003,5 | Beating |
| 35.2 | .000,003,0 | ” |
| 19.4 | .000,003,0 | ” |

### STROPHANTHUS SEED B-566.

| | | |
|---|---|---|
| 18.2 | .000,006.0 | Stopped |
| 20.6 | .000,005,0 | '' |
| 23.6 | .000,005,0 | '' |
| 15.4 | .000,005.0 | '' |
| 43.7 | .000,005,0 | '' |
| 18.1 | .000,004,0 | '' |
| 18.5 | .000,004,0 | '' |
| 25.8 | .000,004,0 | '' |
| 28.0 | .000,004,0 | Beating |
| 28.8 | .000,004.0 | Stopped |
| 34.4 | .000,004,0 | '' |
| 37.2 | .000,003,5 | '' |
| 45.3 | .000,003,5 | Beating |
| 49.0 | .000,003,5 | '' |
| 37.6 | .000,003,0 | '' |

## GUINEA PIG METHOD.

### F. E DIGITALIS NO. 416233.

| Weight of pig in Grammes | Dose per Gramme | Result |
|---|---|---|
| 709 | .000,5 | Recovered |
| 786 | .000,5 | '' |
| 825 | .000,5 | '' |
| 467 | .000,5 | Died |
| 524 | .000,5 | '' |
| 694 | .000,6 | Recovered |
| 701 | .000,6 | '' |
| 814 | .000,6 | Died |
| 835 | .000,6 | '' |
| 744 | .000,7 | '' |
| 517 | .000,7 | '' |
| 481 | .000,8 | '' |

### F. E. DIGITALIS NO. 335929.

| | | |
|---|---|---|
| 750 | .000,4 | Recovered |
| 340 | .000,4 | '' |
| 736 | .000,4 | '' |
| 680 | .000,4 | '' |
| 815 | .000,5 | '' |
| 630 | .000,5 | '' |
| 737 | .000,6 | '' |
| 772 | .000,6 | '' |
| 737 | .000,7 | Died |
| 725 | .000,7 | '' |
| 531 | .000,7 | '' |
| 552 | .000,7 | Recovered |
| 731 | .000,8 | '' |
| 538 | .000,8 | Died |
| 375 | .000,8 | '' |

ONE-HOUR FROG-HEART METHOD. VARIETY RANA PIPIENS. TEMPETATURE 20° C.

F. E. DIGITALIS NO. 416233.

| Weight of frog in Grammes | Dose per Gramme | Result |
|---|---|---|
| 40.9 | .000,50 | Beating |
| 42.9 | .000,60 | " |
| 39.1 | .000,60 | " |
| 34.0 | .000,70 | " |
| 46.5 | .000,70 | " |
| 42.2 | .000,75 | " |
| 50.8 | .000,80 | " |
| 38.8 | .000,80 | " |
| 31.2 | .000,90 | " |
| 38.2 | .000,90 | Stopped |
| 31.4 | .001,00 | " |
| 35.5 | .001,00 | " |

F. E. DIGITALIS NO. 335929.

| Weight of frog in Grammes | Dose per Gramme | Result |
|---|---|---|
| 28.9 | .000,90 | Stopped |
| 22.0 | .000,80 | " |
| 21.0 | .000,70 | " |
| 27.6 | .000,70 | " |
| 23.0 | .000,70 | " |
| 30.5 | .000,70 | " |
| 42.9 | .000,70 | " |
| 19.3 | .000,65 | Beating |
| 20.2 | .000,60 | Stopped |
| 23.5 | .000,60 | " |
| 36.5 | .000,60 | " |
| 37.4 | .000,60 | Beating |
| 36.5 | .000,50 | " |

The assays of these preparations by other methods have been inserted here, with the belief that they will be of some interest to the reader if closely analyzed, although no decided conclusions can be drawn from so small a number. Considering F. E. Digitalis No. 416233 and No. 335929 by the guinea pig and frog-heart methods, it will be seen that while they show almost the same results on the guinea pig, there is a decided difference on the frog's heart. A lack of relationship in the results obtained by these two methods has been observed by others. Remembering that these fluids test the same on the guinea pig, consider the assays by the cat method where No. 416233 is decidedly more active than No. 335929, the reverse of what was found by the frog-heart method.

Attention should be called to the lot of animals used for No. 335929, which was perhaps the least suitable of any. It may be noticed that the greater number were females, varying considerably in size, some being in different stages of lactation. (No. 6 was in the early stage and had exceptionally large glands.) The males were all large and the results, perhaps a coincidence, varied somewhat in relation to the weight.

| | Weight | Result |
|---|---|---|
| No. 2 | 3.18 Kgms. | .140,3 |
| No. 8 | 3.00 Kgms. | .127,8 |
| No. 10 | 2.46 Kgms. | .090,8 |

The assays on the two samples of strophanthus seed are almost identical by the frog-heart method, and show but a small difference by the cat method.

## DISCUSSION.

*Animals.*—Hatcher and Brody selected cats in preference to dogs, and I believe rabbits, for several reasons, namely: "Accuracy afforded, facility with which they may be obtained, ease with which they may be handled, cheapness, and the fact that their use does not affect the sensibilities of the sentimental portion of the community to the same extent that the employment of the dog does." Having used no other animals for this particular method, I cannot remark on the point of accuracy. My experience has been that there is little in their favor regarding cost, all things considered. Cats are easily handled, though to my mind are no more so than dogs, or rabbits, except that in the case of the latter greater care is neccessary in regard to any dissection or the giving of anæsthetics. I have found them far more difficult to obtain than rabbits and hardly less so than dogs. Whether their use affects the sensibilities of the sentimental portion of the community less than that of the dog seems questionable. At any rate, the use of cats certainly does affect the sensibilities of many people, and the procuring of a sufficient number of animals for this piece of work has been the source of considerable trouble. And for a manufacturing plant of this size, to secure enough cats to carry out the routine assays on the several members of the digitalis series, would be a practical impossibility. If some easily procurable animal such as the rabbit could be used for this work, then one great difficulty would be removed. This point is of immense importance to the manufacturer by whom nearly all of the practical physiological assaying will always be done.

Having experienced difficulty in buying cats, an attempt was made at this laboratory to raise them,but this met with poor success. It has seemed that only under the very best conditions can cats be kept well for any considerable length of time. It has been our not infrequent experience that cats will refuse sweet milk and raw beef for some time after having been received, and while an abundance of food has been supplied,our cats have usually lost in weight

When cats are needed for this work they should be made to fast for at least twenty-four hours, as otherwise vomiting will frequently occur, particularly under digitalis. Greater accuracy can also be obtained in regard to the weight.

Lactating animals cannot be depended upon as they seem to posses a greater tolerance for the drug, the degree depending on the stage of lactation.

*The Period and Rate of Injection.* The lethal dose of any of the digitalis bodies cannot, of course, be told at the outset. This is indeed the figure sought. Therefore, "50 per cent. of the lethal dose" is a quantity which can only be widely approximated by one's experience with the given preparation. Whether this point in itself is a matter of great importance, within certain wide limits, I am unable to say. It would seem to be of importance, however, that the injection of all of these drugs be proportioned as evenly as possible over the ninety minutes. After one has injected an amount of digitalis, for example, and has waited the twenty minutes, he is ready to proceed with the ouabain solution. Since he does not know the value of the digitalis, he does not know, consequently, how much ouabain solution it will be necessary to inject during the following period of one hour. And not knowing this point, he is unable to judge how rapidly to inject. If he calculates on 5 c.c. when 10 c.c. would actually be required, then he will come to the end of the ninety-minute period with the animal still alive, and he must cautiously proceed with the probable result that one hundred and five minutes or so will be covered in completing the experiment. And having injected at a slower rate possibly a larger amount of ouabain may have been required. On the other hand, if he calculates on 10 c.c. when only 5 c.c. are necessary, he may kill the animal before the end of the period—perhaps in seventy-five minutes. And having injected at a more rapid rate possibly less ouabain may have been used than would have been under normal conditions.

It might be remarked that the first experiment would furnish these points. This might be true, still, it might happen that the results from number one would be exceptional. Then the operator would be thrown off on number two, and when he found the results from number two quite different, number three would be necessary in order to tell which was more nearly correct.

If these points are of no importance then it would seem that the time limit of ninety minutes would be of no importance.

*Number of Animals and Time.*—In general it would seem that at least three experiments would be necessary in order to determine with confidence the strength of a preparation. If two out of three results checked quite closely, as under F. E. Digitalis No. 405467 (.113,5, .115,2) that number might be sufficient. Under strophanthus seed No. B-565, however, the results show a gradual increase up to the sixth experiment (.001,19, .001,37, .001,53, .001,66, .001,88), and under F. E. Digitalis No. 416233 results Nos. 1, 4 and 6 check each other rather closely (.077,3, .086,1, .087.4), and Nos. 2, 3, and 5 at a different figure check each other even more closely (.065,8, .068,2, .068,8),

If three or four experiments were sufficient then an assay could be made in one day, a point in favor of the method. This would require one person's entire time and attention for the four and a half or six hours, besides part of the time of an assistant. At that more actual time would be required than for any of the other methods.

*Ease of Manipulation and Accuracy.*—The method seems simple, and still all points considered, it is the most difficult of all with which I am acquainted.

My results have been quite disappointing. They show variations for the different preparations, as follows:

| | |
|---|---|
| Oubain | 123.3 per cent. |
| Excluding results Nos. 2, 4, 8, and 25 | 61.4 per cent. |
| Strophanthus Seed No. B-565 | 57.9 per cent. |
| Strophanthus Seed No. B-566 | 48.3 per cent. |
| F. E. Digitalis No. 405467 | 19.0 per cent. |
| F. E. Digitalis No. 416233 | 32.8 per cent. |
| F. E. Digitalis No. 335929 (excluding lactating animals) | 90.5 per cent. |
| Excluding lactating animals and No. 1 | 53.0 per cent. |
| Tr. Digitalis No. 2-B (excluding Nos. 3 and 5) | 67.8 per cent. |

My results with crystalline ouabain would indicate that the lethal dose of this substance varies considerably with different animals. It seems, then, irrational to estimate the value of a preparation of digitalis, from its supposed equivalent of a body which is in itself, for any given animals, an unknown quantity. The authors of this method claim that crystalline ouabain will exactly replace digitalis in regard to its toxicity on the cat. It seems to me, however, that there might be some variance in its power to exactly replace different samples of digitalis depending on the proportion of active principles present and the conditions of these principles, whether or not decomposed. Since the amount of digitalis to be injected which will represent 50-75 per cent. of the required amount is an unknown quantity, it necessarily follows that the amount of ouabain required to complete the experiment, even if its toxicity could be exactly known, is an unknown quantity. Therefore, not knowing the amount of ouabain required, the rate of injection, which probably plays an important part, cannot be known. Lastly, the time required to kill, being dependent on the rate of injection, constitutes another unknown factor. So, when testing a sample of digitalis, one has to deal with six or more unknown factors. This requires an operator of considerable experience and skill.

## SUMMARY.

Considering the results of this work, together with my experience with the other methods, I am led to make the following statements in conclusion:

The cat method of Hatcher and Brody is unquestionably the most complicated and difficult of all the American methods, requiring an operator of considerable experience in animal experimentation.

It is *not* a method that will be found convenient and generally serviceable by the retail pharmacist.

It is more time-consuming than the other methods, requiring constant attention when started.

The item of expense, like that of the guinea-pig method, is decidedly in its disfavor.

The procuring of a sufficient number of suitable animals is a practical impossibility for the manufacturing parmacist having a large number of preparations to test. This may also be the source of much unpleasantness and trouble.

Lactating animals cannot be depended upon as they seem to possess a greater tolerance for the drug, the degree depending on the stage of lactation.

While individual results will not infrequently check each other very closely, considering the results of an entire assay, great variations will often be observed, amounting in some cases to more than 100 per cent.

When testing a preparation one has to consider six or more unknown factors, namely:

1. Toxicity of ouabain.
2. Power of ouabain to exactly replace the digitalis bodies.
3. Amount of digitalis to be injected.
4. Amount of ouabain to be injected.
5. Rate of injection.
6. Time.

This method has perhaps one point of superiorty over all others in that the matter of absorption is entirely eliminated.

DEPARTMENT OF EXPERIMENTAL MEDICINE.

# THE POTENCY OF FIRST YEAR CULTIVATED DIGITALIS LEAVES AS INDICATED BY PHYSIOLOGICAL ASSAY

F. A. MILLER, B. S., AND W. F. BAKER, B. S., M. D.

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The supply of many valuable medicinal plants once so abundant from natural sources is rapidly becoming exhausted. This has been brought about by the destruction of the forests; the devotion of more and more of the waste lands to agriculture, and the destructive methods of harvesting without any thought or care to preservation. The supply was ample and little effort was made toward cultivation. Where attempts were made to introduce medicinal plants into other countries, soil and climatic conditions were often unfavorable. Many plant forms mature very slowly and several years must pass before the drug can be marketed. Possibly from this arose the belief that cultivated plants were less valuable than wild ones. The small returns to be had and the limited demand for the product has been responsible for the undevelopment of this form of industry.

In view of the advance now being made in plant culture and improvement it is entirely within the realm of possibility that the desired constituents of drug plants can be increased and undesirable ones decreased or eliminated as is being done with many cultivated forms. In the case of some drugs, the active principles of which can be more or less closely paralleled by synthetic preparations, it may be questionable as to the advisability of expending much energy upon their cultivation.

The forms which contain neutral principles, glucosides, etc., cannot be so closely paralleled. In this case digitalis easily stands first. One of the factors in the way of its successful cultivation has been the belief that wild plants were more potent, and this has been furthered by the Pharmacopeias of various countries. Some of these Pharmacopeias now admit cultivated leaves, but all require the second-year leaves at the time of flowering. This requirement appears to be founded more upon tradition than as a result of scientific investigation. This is a question of considerable economic importance. A larger quantity of leaves can be obtained from first year plants, and if it can be conclusively proven that first year leaves are as therapeutically active as the second, it will greatly shorten the period and the cost of production.

From a review of the literature it appears very little work has been done to determine the relative potency of first and second year leaves.

Duffield[1] in 1869, using chemical methods, found American leaves superior to English and these in turn superior to German. Edmunds[2] assayed, biologically, three U. S. P. tinctures made from English leaves and three from German leaves and found them to give values of 8-18-25 and 11-20-29 respectively. Ott[3] pointed out that Bohemian leaves were much more toxic than others.

Focke[4] found cultivated leaves to be 50 per cent. less active than leaves from wild plants. Allen's English leaves are garden grown and are recognized as quite active.

Hart[5] found first year's leaves to be 20 per cent. more toxic than second year. Hale[6] reports some assays of first year garden-grown leaves, one sample grown at Arlington, Va., in 1907, a second 1909, and a third sample grown at Madison, Wis., in 1908. Each lot of leaves was reduced to a No. 60 powder and made into tinctures according to to the U. S. P. VIII. The M. L. D. per gram body weight by the one hour frog heart method, for Arlington, 1907, was 0.0050 cc.; for the 1909, 0.0050 cc., and for the Wisconsin, 1908, 0.0055 cc. These were compared with a tincture made at the same time from selected English leaves of second year's growth. The M. L. D. for this was 0.0070 cc. He further reports a comparison of a sample of first year garden-grown with a sample of wild-growing second year leaves from Seattle, Wash. The first year leaves assayed 0.0060 and the second year leaves 0.0085.

In view of these statements and of the approaching pharmacopeial revision it has been deemed advisable to publish the results of some assays of samples of first year cultivated leaves and compare them with samples of selected commercial leaves. The samples tested were taken from plants grown from seed purchased of Henry A. Dreer of Philadelphia. The botanical source is Digitalis gloxineaeflora mixed. It is a well known fact that this form is a cultivated and improved gloxinia-like strain of the official Digitalis purpurea L. It is probably one of the oldest of the cultivated varieties of the genus Digitalis and for this reason should represent the average conditions which are to be expected in cultivated forms of this group. The seeds were planted June 10th, 1911, and soon after germination were transplanted to two-inch flower pots. They were retained in these until the latter part of August of the same year when they were transplanted to a bed in the conservatory maintained at this laboratory.

The bed was filled with a mixture of equal parts of clay loam and decayed vegetable substance with an admixture of a small amount of sand. The plants were grown continuously in this situation without the addition of any plant food until the second of February, 1912, when the leaves were collected for testing. The plants at this time appeared as large rosettes of luxuriant leaves. They were in perfect condition and represented a considerable range in leaf variation as noted in about fifty plants. These plants, including the three tested, have been under observation and continuous cultivation throughout their entire existence and have responded perfectly to regular methods of cultivation. The leaves which were collected for testing consisted of about an equal mixture of fully developed and half-grown leaves. These when collected were designated as samples B-994, B-995 and B-996. They were placed in a drying oven and the temperature slowly raised to 100° C. Drying was considered complete at the end of twenty-four hours. During an interval of twelve hours the temperature was lowered to 60° C. The leaves after being dried were reduced to a No. 60 powder and exhausted with 75 per cent. alcohol. At same time and by same method tinctures were made from samples of commercial leaves designated as No. 1, 2 (cultivated) and 3. The alcoholic strength was reduced to 25 per cent. before testing. Ten cc. of these tinctures represented 1 gm. of drug. These samples were all assayed at the same time by the one hour frog heart method at 20° C.

The results were as follows:

| Sample | M. L. D. per gm. body weight. |
|---|---|
| B-964 | 0.0050 cc. |
| B-995 | 0.0040 cc. |
| B-996 | 0.0030 cc. |
| No. 1 | 0.0040 cc. |
| No. 2 (cultivated) | 0.0030 cc. |
| No. 3 | 0.0025 cc. |
| Ouabain 1:10000 | 0.00000045 gm. |

These results, although not conclusive, indicate that leaves from horticultural varieties are by no means inactive; that leaves collected prior to the flowering period may be nearly or quite as active as second year leaves from the wild plants and that conservatory conditions do not materially lessen the activity. There also appears to be a marked variation in the activity of the individual plants. This condition if existent in commercial digitalis leaves may account in part at least for the variation that has been found to exist, both in the crude drug and its preparations. Whatever influence this individual variation may have upon the character and quality of the drug, or whether it may ever be utilized in bringing about greater uniformity, are matters of conjecture. This condition of variation, however, suggests a broad field for further investigations in the improvement of medicinal plants. If the active constituents of digitalis were found to be of an inherent nature a practical application of the methods of plant selection and breeding would be possible for the purpose of improvement. In this connection it would be of great importance if the leaves of first year plants of cultivated varieties could be utilized. These forms have already passed through the experimental stage which is always necessary to the successful introduction of any plant forms and would thus respond more readily to methods of breeding. The preliminary nature of this work, however, will not permit of a full discussion of the possibilities in drug plant improvement. It is sufficient to say that work of this nature has been undertaken in connection with the further testing of the many different species and varieties of digitalis. Seeds of these forms have been obtained not only from all the prominent sources of this country but also from those of England, Germany and Japan.

Individual plants of the various forms from these sources will be tested at different periods of growth and at different seasons. The three plants herein considered will be continued under cultivation and again tested at the time of flowering. At this time these plants will be inbred and the seeds collected separately. The following year the progeny will be grown and tested separately for relative values and uniformity.

[1] Duffield: Am. Jr. of Pharm., 1869, XLI, 55.
[2] Edmunds: Jr. A. M. A., 1907, XLVII, 1744.
[3] Focke: Arch. der Pharm., 1903, CCXLI, 128.
[4] Hale: Bull. No. 74, Hyg. Lab. U. S. Pub. Health and Marine Hospital Service.
[5] Hart: Pharm. Jr. and Tr., 1908, XXVI, 440.
[6] Ott: Verhandl. d. Cong. f. innere Med. Wiesb. 1901.

*Departments of Botany and Experimental Medicine.*

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# HOW FAR VARIATIONS IN FROGS CAN BE OBVIATED BY THE USE OF OUABAIN

W. F. BAKER, B. S., M. D.

It is well recognized that uniformity in strength of remedial agents is one of the most important factors in scientific medicine. It is equally well recognized that one of the most important groups in the whole materia medica, viz., digitalis and its allies, varies widely, 1 to 400 per cent., according to some investigators, not only in the crude drug and galenical preparations but in some of the so-called pure principles. For the above reasons a vast amount of work has been done in the endeavor to perfect a method by which these important agents may be assayed and standardized.

Most of the drugs which contain alkaloids and some of those which contain a single physiologically active glucoside can be assayed and standardized very readily by chemical methods. In the case of digitalis the activity of the drug is not represented by one but by several active principles, all of which cannot as yet be accurately assayed by chemical methods as demonstrated by Barger and Shaw.[1] Until it can be shown that these active principles are present in a definite ratio it appears very irrational to assume that such preparations can be standardized on the basis of a single active principle as advocated by some, since the activity of the drug is represented by the sum total of activity of these principles.

Physiological testing had been used for years in a qualitative way, but it remained for Houghton[15] in 1898 to propose it for the production of uniform preparations. After experimenting with various animals he decided upon frogs as being the most suitable for the purpose and while the frog continued to be the favorite animal the method as proposed by Houghton has undergone several modifications.

Two other type methods with their various modifications have been proposed and adopted by some for assay of the heart tonic preparations.

The blood pressure method while a good qualitative test cannot be used with any degree of accuracy to standardize the digitalis series owing to the slowness with which the effects of the initial dose disappears. The lethal dose method on the guinea pig, as originated by Laborde[17] and so warmly advocated by Reed and Vanderkleed,[20] is open to the objection that other constituents than those desired may be present and produce death. A preparation containing considerable decomposition products would appear to be an acceptable preparation while it would in fact be a very dangerous one. Another objection, as pointed by Edmunds and Hale[8] and also by Nestor, the cause of death in the case of mammals is not due in every case to the action upon the heart but upon the medulla, and for these reasons they think that methods which employ as a standard the minimum lethal dose obtained upon higher animals are not applicable to the physiological assay of the digitalis series. Hatcher's modification of the lethal dose method is undesirable for like reason, for its difficult technic, and lack of uniformity of results as demonstrated by Eckler.[6]

Logically the most desirable method is that one in which the end point will be exerted upon that tissue or organ upon which its therapeutic effect is desired, which shall be as free as possible from technical difficulties, and can be carried out at a moderate cost.

The frog method fulfills all these desirable qualifications. Dixon has pointed out that the principle difference in action on man and lower animals is the difference in action of those drugs which act upon the central nervous system. Agents which act upon one tissue or organ will act in like manner upon this same tissue or organ in any other animal.

The frog method as proposed by Houghton is essentially a lethal dose method and is open to the objection of all lethal dose methods. Another objection to this method is that twelve hours cannot be gotten into a working day. I have not infrequently found that frogs which had received the M. F. D. of digitalis for the one-hour method were active at the end of the twenty-four hours, but when opened it was found that the heart had stopped in systole.

Barger and Shaw,[1] Famulener and Lyons,[10] Edmunds and Hale[8] and others have compared the results of the frog heart method of assay with the results obtained by chemical assay and find the results more reliable and consistent by the physiological methods.

Moskowitsch's[18] results led him to believe that the physiological method while not necessarily useless is very unreliable. Focke[11] concludes from his results by observing proper test conditions and using a standard the method is very valuable and reliable.

Not withstanding the points of superiority of the frog heart method for the assay and standardization of the heart tonic group the Committee on Physiological Assay of the Philadelphia Branch of the Am Ph. Assoc. report[23] they are inclined to prefer the test upon the guinea pig, although they admit that the work of Edmunds and Hale[8] gives more accurate results, showing in no case an error of more than 10 per cent. They state there are two serious objections to the frog method as an official process of assay, viz., the factor of the time limit and what appears to them an almost unsurmountable objection, the great variation in the susceptibility of frogs due to difference in species, season, temperature, locality and the limited range of certain species. The only method, the report continues, of overcoming these obstacles would seem to be by comparing the preparations tested with a standard. "We are convinced," they say, "that such a method of standardizing is undesirable for pharmacopœial purposes because it would necessitate a distribution either by the Government or the Pharmacopœial Convention of a standard. "Guinea pigs," they say, "are obtainable in all parts of the world, and temperature, food, season, weight and sex do not influence their reaction." Experiments in this laboratory, however, have clearly demonstrated that guinea pigs do vary in their susceptibility almost as much as frogs and for this reason require a standard the same as frogs. In this laboratory we have found it impossible to the present time to secure enough guinea pigs to do our routine work, and from the reports which have come to us from time to time I believe that others are having the same difficulty.

It was with the view to clearing up some of these points on the variation in the susceptibility of frogs that I have undertaken a review of the literature and carried out a series of experiments to determine the influence of some of these factors.

Dixon[5] says that frogs do not vary as much as 50 per cent. during the year, being the most vigorous in the summer, least in spring. Famulener and Lyons[9] say, "We may be sure they will show far less variability than warm-blooded animals, and out of a dozen seemingly healthy frogs not more than one or two will show a variation of as much as 10 per cent. below or above the average susceptibility."

Hale[12] says the frog method gives all that any assay method can give and by using a digitalis preparation of known activity and keeping quality, crystalline digitoxin or ouabain proposed, accurate results may be obtained without reference to season, age, sex, temperature or species of frog used. He further pointed out that the unknown factors of absorption, elimination and individual susceptibility will persist in all attempts on assaying members of digitalis series. These, however, may be eliminated by using a large number of animals, discarding those which give too widely diverging results and taking the average of a number of concordant results.

Other factors of equal importance, such as age: size, sex, season' temperature, etc., can be eliminated or controlled when once their import is known. All recognize the necessity of using healthy frogs, and as Hale remarks, it is not difficult to obtain and keep them so.

Nearly all investigators have expressed the desirability of using a certain species of frogs, but Prevost,[9] Focke,[11] and Vanderkleed[22] appear to be the only ones who have carried out any investigation to determine just what influence species might have on the susceptibility and their results show considerable difference in the susceptibility of the different species but for the same species the susceptibility was fairly constant. This factor, however, is one easily controlled; Houghton says any species may be used provided all preparations of one assay are tested on the same species.

Bührer[2] appears to be the first to make any mention of the influence of sex and employes females exclusively; Vanderkleed[22] claims as a result of his experiments females are 19 per cent. more resistant than males of same species and from the same locality. Barger and Shaw,[1] Dixon[7] and Ziegenbein[23] used only males while Focke[11] and Edmunds and Hale[8] believe sex plays a very inferior part. This accords with my own experience unless the females contain a large egg mass when they appeared to be slightly more resistant. To avoid any influence sex might have, I use the females for making preliminary tests and determine the end point on male frogs.

The early investigators disregarded the factor of weight, or, if they selected frogs of about equal weight, did not consider it in their calculations. Houghton advises using frogs varying not more than three grams in weight. Ziegenbein[23] used frogs weighing about 25 grams, but did not think a few grams made any difference. Focke[11] uses frogs weighing 20-30 grams and considers the weighing important. Moskowitsch[18] takes but little account of weight in calculating his results and this may account for the great variations in his results. Prevost[19] at first disregarded weight, but later weighed his frogs. He believed large frogs less sensitive than smaller ones, still he believed that *volume* of less import for digitalis than for aconite.

As to the effect of age or the length of time in captivity workers differ: Bührer's[2] experience led him to believe they varied considerably in two weeks' time to one and the same drug.

Ziegenbein[23] used only freshly caught males. Focke[12] did not notice any appreciable difference at same temperature of frogs recently caught and those kept in captivity for several months. Houghton[15] and Hale[12] believe frogs vary but little if kept cool and have access to running water, and in the main this has been my experience. When the frogs first arrive they are not very resistant, but after a week or ten days do not vary greatly.

Season appears to play a greater part than captivity: Fagge and Stevenson[10] and Ziegenbein[23] concluded season made but very little difference. Focke[11] thinks frogs should be caught not earlier than the end of June and advises the examinations to be made in July, August and September, during which time the susceptibility does not vary greatly, while in the spring the average values are considerably lowered. Dixon[5] finds frogs most active and vigorous and more resistant in summer, least in spring. Moskowitsch[18] found July frogs more resistant in July than in winter. Focke[11] was first to call attention to the influence of temperature and in fact appears to be the only one who has carried out any investigation to determine just what influence temperature changes might have. Hale[12] says the temperature is a primary and important essential and gives it as one of the precautions to be observed in using the frog as an assay animal. He advises making all assays at 22° C., this being ordinary room temperature and the one most easily maintained. And while a slightly higher temperature might not be inconsistent with accuracy on account of the easy susceptibility of frogs to heat lower temperatures are to be preferred to high. The committee of the Philadelphia Branch of the Am. Ph. Assoc'n reply to Dr. Hale's discussion before the Philadelphia Branch in which he made the statement that the difference due to temperature may be overcome by working always in a room of certain temperature; by saying, "We are not familiar, however, with any experimental proof of this statement, and it seems a priori improbable."

Very little work appears to have been done to determine the effects variation of temperature would have upon the susceptibility of frogs and in our laboratory, one of us, Dr. Haskell, convinced that the variations in temperature played a considerable part in the susceptibility of the frogs, injected a series of frogs in the still room where the temperature was very high. He found that the M.L.D. by the one-hour method was considerably less than at ordinary room temperature; following this, I decided to carry out a series of experiments to determine more fully the effects of temperature on the susceptibility of the frogs.

All our frogs with the exception of one lot come from Illinois.

As soon as they arrive they are unpacked and placed in the frog cellar in galvanized iron tanks. These tanks are about two feet square and six inches deep, being fitted with a wire net cover. The tanks are slanted so that running water may cover about one-half the cage bottom. The temperature in this cellar did not go above 15° C. during the time these experiments were in progress. After unpacking, the frogs are allowed to remain in this cellar at least ten days before being used. They are brought from the cellar to the operating room in such quantities as are required, carefully weighed, and placed in individual cages in a thermostat where they are allowed to remain for an hour at the temperature at which it is desired to operate before they are injected. The doses are calculated per gram body weight, measured out and diluted to 0.015 c.c. per gram body weight. Five doses are prepared and injected quickly so that each series may be subjected to as near identical conditions as possible. The method is the one-hour frog-heart method suggested by Cushny and advocated by Famulener and Lyons.

Just before the end of sixty minutes the frogs are pithed, brain and cord and heart exposed. The complete cessation of the ventricle in systole, with the auricle still beating or responding to stimulation, is taken as the end point. If, when the lymph sac is opened there is any excess of moisture present, the result is noted as non-absorption and the experiment is not considered in drawing conclusions. The females are used for preliminary tests and the end point determined on selected male frogs.

A protocol of the first experiment is given below:

1-12-12. Ouabain 1:10000 in 25 per cent. alcohol. Frog lot 246.

15° C.

| Weight of frog | Dose in gms. per gm. body weight | Results |
|---|---|---|
| 27.3 | 0.0000008 | —Beats |
| 21.8 | 0.0000008 | —Beats |
| 25.5 | 0.0000009 | —Beats |
| 28.4 | 0.0000009 | + Stopped |
| 22.5 | 0.0000010 | —Beats Nonabs |
| 22.7 | 0.0000010 | + Stopped |
| | M.L.D. 0.0000010 | |

20° C.

| Weight of frog | Dose in gms. per gm. body weight | Results |
|---|---|---|
| 20.0 | 0.0000005 | —Beats |
| 28.2 | 0.0000006 | —Beats close |
| 28.3 | 0.0000006 | + Stopped |
| 26.1 | 0.0000007 | + Stopped past |
| 25.6 | 0.0000008 | + Stopped past |
| 20.4 | 0.0000008 | + Stopped past |
| | M.L.D. 0.0000006 | |

| Weight of frog | Dose in gms. per gm. body weight | Results |
|---|---|---|
| | 30° C. | |
| 26.2 | 0.0000002 | —Beats |
| 20.8 | 0.0000003 | + Stopped |
| 22.8 | 0.0000004 | + Stopped past |
| 24.6 | 0.0000004 | + Stopped past |
| 27.0 | 0.0000005 | + Stopped past |
| | M.L.D. 0.0000003 | |

Variations in temperature appears to be a very important factor in the susceptibility of frogs, their resistance decreasing as the temperature increases; subsequent experiments not only substantiated the above results but demonstrated the variation in susceptibility of different lots of frogs.

The results emphasize the necessity of using a preparation to standardize the frogs for each series of assays. Just what this standard should be workers differ. To be of any value it must be one that will exactly represent and parallel the activity of the preparation to be tested and must be of good keeping quality. Focke[12] advocates properly prepared and preserved digitalis leaves, making an infusion for testing. Hale, however, has demonstrated the variation which may occur in preparations made from the same lot of leaves, due probably to faulty technic in the process in extraction. Tincture of digitalis has been suggested, but the work of Houghton and Hamilton and that of Hale demonstrates the deterioration which may take place and the inadvisability of using a galenical preparation for the standard notwithstanding Houghton's results with Trs. of Strophanthus: Houghton recommends Kombe strophanthin and Hale suggests ouabain or crystalline digitoxin, either of which are definite chemical substances and so far as is known are stable.

In order to determine whether the susceptibility of frogs varied concomitantly for Ouabain and Strophanthus, a 5 per cent. and a 10 per cent. Tr. of Strophanthus bought on open market were assayed along with a 1:10000 solution of Ouabain in 25 per cent. alcohol, the tinctures being diluted so as to bring the alcoholic strength to 25 per cent.

The results are given in the following table:

| | Ouabain 1:10000 | Tr. Strophanthus 10 per cent. | Tr. Strophanthus 5 per cent. |
|---|---|---|---|
| 10° C. | 0.0000008 | 0.00008 | 0.00020 |
| 20° C. | 0.0000004 | 0.00005 | 0.00012 |
| 30° C. | 0.0000002 | 0.00002 | 0.00006 |

The variation for Ouabain and Strophanthus therefore are very closely parallel.

Having received about this time a small consignment of frogs (averaging about 50 grams) from Minnesota, I used them in this set of experiments with the following results:

| | Ouabain 1:10000 | Tr. Strophanthus 10 per cent. | Tr. Digitalis 10 per cent. |
|---|---|---|---|
| 10° C. | 0.0000010 | .00017* | .006* |
| 20° C. | 0.0000008 | 0.00009 | .004 |
| 30° C. | 0.0000003 | .00004 | .003 |

---

*I did not have enough frogs to complete the end point for these two. The dose given was the smallest dose administered and was fatal.

As in the previous experiments the variation is closely parallel for Ouabain and Strophanthus, but the digitalis did not show the same ratio of variation. In order to investigate this more fully the experiment was repeated, using frogs weighing 15 to 20 grams, with the following results:

| | Ouabain 1:10000 | Tr. Strophanthus 10 per cent. | Tr. Digitalis 10 per cent. |
|---|---|---|---|
| 10° C. | 0.00000080 | 0.000080 | 0.0040 |
| 20° C. | 0.00000045 | 0.000040 | 0.0040 |
| 30° C. | 0.00900020 | 0.000025 | 0.0030 |

By these results I believe I have demonstrated the necessity of using some preparation to standardize the frogs for each assay.

I have demonstrated that while Ouabain or Strophanthus preparations may be used to standardize Strophanthus, they are unsuitable as standards for digitalis and its preparations. For this reason it may be inadvisable to use any of the Strophanthus or digitalis preparations to standardize Apocynum, Convallaria or Squill.

Hale has recommended crystalline digitoxin to standardize digitalis and I have already begun a series of experiments with the glucosides of digitalis and its allies with reference to a standard for each. These will be assayed by both the one-hour method of Cushny and the twelve-hour method of Houghton.

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# SEASONAL VARIATIONS IN THE RESISTANCE OF GUINEA PIGS TO POISONING

C. C. HASKELL, A. B., M. D.

The studies of Hunt[1] in the variations of the susceptibility of animals to poisoning by acetonitrile according to season and diet gave results which are suggestive in several fields. Not the least important of these is the matter of standardization of remedial agents.

It has long been assumed that guinea pigs were especially suitable for the standardization of diphtheria antitoxin. Probably on this account, Reed and Vanderkleed[2] suggested their employment in the attempt to standardize the drugs of the digitalis group. These authors recognized the fact that the commonly used frog varied much in its susceptibility to digitalis poisoning, and they hoped to avoid the trouble and doubt necessitated by the employment of some standard preparation, by which means it is hoped that the variations in susceptibility can be rendered unimportant.

To use guinea pigs in such a way, however, makes it necessary to assume that the resistance of these animals to digitalis poisoning is always practically the same, regardless of weight, sex, diet, or season. Vanderkleed[3] has shown that age, weight and sex do not influence the susceptibility, and in this we agree. Fully as important, however, are the factors of diet and season. From our earlier work[4] it was evident that diet did influence the resistance of the animals markedly, those on green food requiring a larger dose of the poison than those fed almost exclusively on dry oats. It is not probable that guinea pigs will ever be fed on exclusive green food diet when they are kept for commercial standardization, so our experiments represent an exaggeration of conditions apt to exist. Nevertheless, the variation brought out in so short a time is very suggestive, and seems to indicate the danger of relying upon the animals without the use of a standard drug.

From a continuation of the work which I undertook with Mr. Walters last August,[4] it seems that season also plays a most important role in affecting the resistance of guinea pigs to poisoning by ouabain.

On July 24, 1911, a stock solution of 1 10 per cent. ouabain in 70 per cent. alcohol was made up, and this solution has been employed throughout. As the dose necessary to cause the death of guinea pigs increased, the question arose as to whether this solution had deteriorated. To answer this, seven control solutions were made at various times by three separate individuals from three lots of Merck's ouabain and the strength of all eight solutions showed no differences in strength that I could detect. It would seem, contrary to what is commonly believed, that ouabain in alcohol is quite stable. Nevertheless, it was deemed safer to await a decrease in the resistance of the animals before reporting the results. The stock solution was diluted 1 to 10000 ouabain in 25 per cent. alcohol just before injection. The Hitchens syringe was used throughout and the attempt was to place the drug just under the skin of the abdomen. The animals were observed over a period of 24 hours, any living at the end of that period were considered as having survived the dose.

The lethal dose was determined for the eight months from August, 1911, to March, 1912, inclusive. Owing to the difficulty of securing animals in sufficient numbers, the determination of the lethal dose was not always made on the same date of each month. The animals were kept under similar conditions on a diet of oats, clover hay and cabbage.

The following tables give the results secured:

DOSE IN GRAM PER GRAM GUINEA PIG

| Month | Survived | Died | Minimum Lethal Dose |
|---|---|---|---|
| August | .00000020 | .00000026 | |
| | .00000023 | .00000027 | |
| | .00000024 | .00000027 | .00000028 |
| | .00000025 | .00000028 | to |
| | .00000025 | .00000028 | |
| | .00000025 | .00000028 | .00000030 |
| | .00000025 | .00000029 | |
| | .00000026 | .00000029 | |
| | .00000027 | .00000029 | |
| | .00000027 | .00000029 | .00000028 |
| | .00000027 | .00000029 | to |
| | .00000027 | .00000030 | |
| | .00000028 | .00000030 | .00000030 |
| | .00000028 | .00000030 | |
| | .00000028 | .00000030 | |
| | .00000028 | .00000030 | |
| | .00000029 | .00000031 | |
| | .00000029 | .00000033 | |
| | .00000029 | .00000035 | |
| | .00000029 | .00000035 | |
| | .00000030 | .000000375 | |
| | .00000030 | | |

| | DOSE IN GRAM PER GRAM GUINEA PIG | | |
|---|---|---|---|
| Month | Survived | Died | Minimum Lethal Dose |
| September | .00000022 | .00000028 | .00000030 |
| | .00000022 | .00000029 | |
| | .00000024 | .00000029 | |
| | .00000026 | .00000029 | |
| | .00000028 | .00000029 | |
| | .00000028 | .00000029 | |
| | .00000028 | .00000029 | |
| | .00000028 | .00000029 | |
| | .00000028 | .00000029 | |
| | .00000028 | .00000030 | |
| | .00000028 | .00000030 | |
| | .00000029 | .00000030 | |
| | .00000029 | .00000030 | |
| | .00000029 | .00000032 | |

| | DOSE IN GRAM PER GRAM GUINEA PIG | | |
|---|---|---|---|
| Month | Survived | Died | Minimum Lethal Dose |
| October | .00000028 | .00000036 | .00000036 |
| | .00000029 | .00000036 | |
| | .00000030 | | |
| | .00000030 | | |
| | .00000031 | | |
| | .00000031 | | |
| | .00000031 | | |
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| | .00000035 | | |
| | .00000035 | | |

| | DOSE IN GRAM PER GRAM GUINEA PIG | | |
|---|---|---|---|
| Month | Survived | Died | Minimum Lethal Dose |
| November | .00000036 | .00000036 | .00000052 |
| | .00000036 | .00000045 | |
| | .00000036 | .00000046 | |
| | .00000036 | .00000046 | |
| | .00000038 | .00000048 | |
| | .00000038 | .00000050 | |
| | .00000038 | .00000050 | |
| | .00000038 | .00000050 | |
| | .00000038 | .00000051 | |
| | .00000040 | .00000052 | |
| | .00000040 | | |
| | .00000040 | | |
| | .00000045 | | |
| | .00000046 | | |
| | .00000046 | | |
| | .00000046 | | |
| | .00000046 | | |
| | .00000046 | | |
| | .00000050 | | |

| | DOSE IN GRAM PER GRAM GUINEA PIG | | |
|---|---|---|---|
| Month | Survived | Died | Minimum Lethal Dose |
| November | .00000050 | | |
| | .00000050 | | |
| | .00000050 | | |
| | .00000050 | | |
| | .00000050 | | |
| | .00000050 | | |
| | .00000050 | | |

| | DOSE IN GRAM PER GRAM GUINEA PIG | | |
|---|---|---|---|
| Month | Survived | Died | Minimum Lethal Dose |
| December | .00000048 | .00000049 | .00000052 |
| | .00000048 | .00000052 | |
| | .00000049 | | |

| | DOSE IN GRAM PER GRAM GUINEA PIG | | |
|---|---|---|---|
| Month | Survived | Died | Minimum Lethal Dose |
| January | .00000045 | .00000052 | .00000052+ |
| | .00000045 | .00000052 | |
| | .00000050 | .00000052 | |
| | .00000050 | | |
| | .00000050 | | |
| | .00000052 | | |
| | .00000052 | | |

| | DOSE IN GRAM PER GRAM GUINEA PIG | | |
|---|---|---|---|
| Month | Survived | Died | Minimum Lethal Dose |
| February | .00000025 | .00000035 | .00000035 |
| | .00000030 | .00000040 | to |
| | .00000030 | .00000040 | .00000040 |
| | .00000030 | .00000040 | |
| | .00000030 | .00000050 | |
| | .00000035 | | |
| | .00000035 | | |

| | DOSE IN GRAM PER GRAM GUINEA PIG | | |
|---|---|---|---|
| Month | Survived | Died | Minimum Lethal Dose |
| March | .00000030 | .00000030 | .00000036 |
| | .00000030 | .00000030 | |
| | .00000033 | .00000030 | |
| | .00000033 | .00000033 | |
| | .00000033 | .00000036 | |
| | .00000036 | .00000036 | |

From an examination of these tables, it is evident that in assaying a preparation of the digitalis group upon guinea pigs a standard preparation is as necessary as is the case with frogs. Furthermore, it would also appear that the individual variation of guinea pigs is by no means inconsiderable. The utmost care was used in giving the injections, and when there was any suspicion that the needle had penetrated muscle, that animal was discarded. The variation due to individual peculiarities is shown well in the November series and is also apparent in the March. In the first five pigs injected during this latter month, three succumbed to a dose of

.00000030 gm. per gm. body weight. From this, it might be inferred, that the lethal dose was near .00000030 gm., but when the dose was increased to .00000033 gm., one pig succumbed and three survived. The animals all seemed sound and had been kept under precisely similar conditions.

It is of interest to compare the seasonal variations of guinea pigs to poisoning by various agents. This comparison is better appreciated from an examination of Chart I.

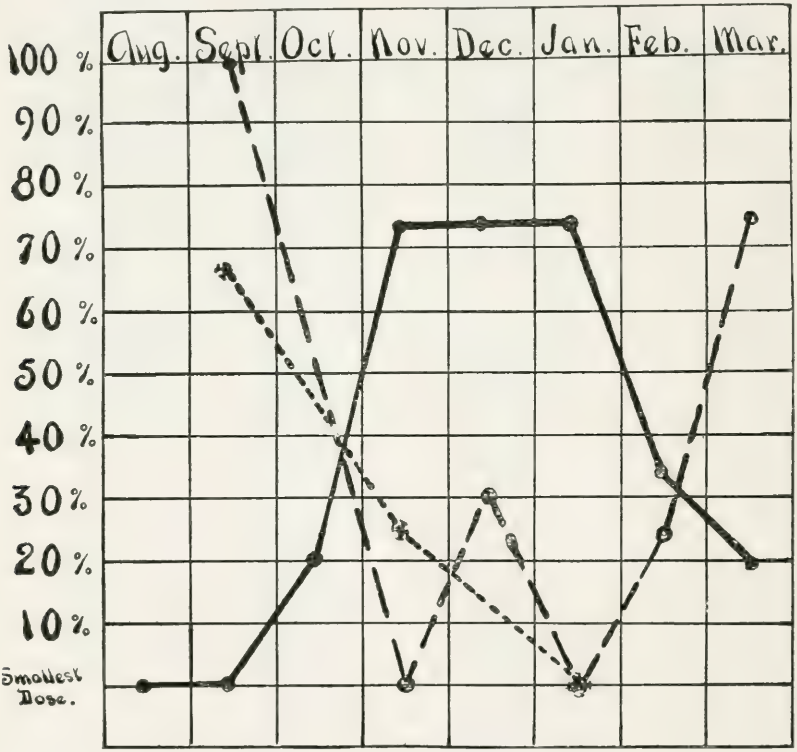

Chart I shows the seasonal variations of guinea pigs to poisoning by acetonitrile, diphtheria toxin, and ouabain. The circles connected by interrupted lines represent the variation to acetonitrile according to Hunt.[1] The crosses and dotted lines, Sudmersen and Glenny's[3] doses of diphtheria toxin. The points and solid lines, the doses of ouabain.

While the curve for the lethal dose of acetonitrile and diphtheria toxin show fairly close agreement, the largest dose of each being required in September and the smallest dose in January; the curve of ouabain is widely different.

This suggests the question, do all the members of the digitalis group show the same curve? This is of the utmost importance, because upon such agreement depends the value of a standard. It is to be hoped that the wide difference between the pharmacology of the bodies charted is sufficient to account for the divergent curves, while the members of the digitalis group, being so closely allied, will show no such variation.

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# THE ADULTERATION OF CASCARA SAGRADA

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[Read before the Scientific Section of the American Pharmaceutical Association at the meeting held in Denver, Col., August, 1912, and reprinted from the Jour. Am. Pharm. Ass'n., November, 1912]

The quality of commercial cascara bark has been so uniformly high for the past few years that little has been said concerning adulterations and inferior grades. Destructive methods of collection have continued, however, and the centers of supply have shifted rapidly to new districts, facts which augment the possibilities of new adulterations and admixtures. Such possibilities exist in the case of most vegetable drugs and an uninterrupted period of uniformity and high quality should not result in a lack of vigilance on the part of crude drug inspectors. That a close inspection of all commercial packages is essential to successful manufacturing operations is evident when the conditions controlling the collection of these products are carefully considered. This is especially true in this country where a considerable proportion of the crude drugs is gathered and prepared for the market by ignorant collectors. They are responsible not only for many adulterations but also for a great many of the variations which regularly appear, the effects of which are always evident in the finished product. Many variations in these products could be and in numerous cases have been traced to certain abnormalities in the crude material.

Such conditions as these have existed in the case of cascara sagrada and the available information indicates that the markets have not always supplied a uniform drug of definite origin. A warning has recently become current that much of the commercial bark is now composed of Rhamnus californica Esch. This has led to an examination of samples purchased from various sources as well as a closer inspection of all shipments of the drug recently received. The method of inspection used in this instance must of necessity be one which will eliminate the adulterant with a minimum amount of time and labor. A review of the work that has been done on the identification of the bark of different species of cascara reveals a difference of opinion on certain important characters. This difference is most evident in the statements referring to the relative width of the medullary rays in Rhamnus purshiana, D. C., and Rhamnus californica Esch. Rusby[1] states that they commonly consist of two rows of cells in purshiana and three or more in californica. He accompanies this statement with drawings by Kraemer which show the rays from one to three cells wide in purshiana and from three to five in californica. Sayer[2] makes no statement as to the width of the rays but includes drawings of both species. These drawings indicate that the rays in purshiana are all two cells wide and in californica from two to four. Kraemer[3] gives the width as being from one to two cells in purshiana and from three to five in californica. It is to be noted that Kraemer furnished the drawings for Rusby[1] in which the width is shown to be from one to three cells in purshiana. Dome and Engelhardt[4] classify the rays in purshiana as usually bicellular. Moeller[12] says they are composed of two or three rows of cells. In the examination of the commercial samples above mentioned (five from New York and three from San Francisco) as well as in numerous shipments of the drug, eighty-five per cent. of the microscopic inspections have shown the medullary rays to be from one to three cells wide. Rays from one to two and from one to four cells wide have been noted in only nine per cent. of the examinations, while those from one to five and from one to six have not exceeded one per cent. It may be stated from this, that if the inspection of cascara bark for the isolation of Rhamnus californica Esch., can be based upon the relative width of the medullary rays, as stated by two authorities, the commercial conditions at present are not alarming.

The most recent report of adulterated cascara bark comes from abroad, in which Perrot[5] says that powdered cascara sagrada is sometimes adulterated on the continent of Europe with powdered frangula. The small amount of the drug handled in this form in America, however, eliminates the possibilities of a similar occurrence. Also its suggestion as an adulterant in the crude form by Beckett[6] does not seem well founded when the very distinct nature of the two barks is carefully considered. Its presence in cascara could readily be detected upon a basis of macroscopic characters alone. Not so, however, with certain other forms that have appeared. Earlier reports indicate that the bark has varied considerably.

In 1888 Moss[7] called attention to a spurious cascara which though he was unable to identify he felt sure was Rhamnus purshiana D. C. collected out of season. The following year Squibb[8] discussed the general unsatisfactory condition of commercial cascara as it occurred during the summer of 1889. He accounts for this condition as being due to different methods of collecting and curing. Further complaint is not made until 1905 when Zeig[9] in a discussion of cascara bark—its habitat, collection, supply, etc., ascribed the difference in the appearance of the crude article to climatic influences and manner of drying. Other evidence is at hand which points to actual substitution and adulteration. Rusby[10] 1890 claimed to have found this to be the case to the extent of car load lots. He attributes the cause to Rhamnus californica Esch. This hardly seems consistent with a statemenl made the previous year[11] which appeared as a criticism of the article by Moss. Referring to the inferior condition of cascara, he says that it is not so much a question of kind, inasmuch as Oregon and California barks are practically of the same species and variety. He also states that he had been able to identify the bark Cornus nuttallii Audub. as a cascara substitute. Beckett[6] characterized the market conditions for a few years prior to 1889 and believed that three species were collected and marketed indiscriminately, i. e. Rhamnus californica Esch., Rhamnus purshiana D. C. and Rhamnus croceus Nutt. Concerning their comparative appearance he says they are almost identical. Another substitute is mentioned by this author which he classified as some species of alder and which resembled true cascara in appearance and taste.

From the foregoing it is evident that inferior cascara sagrada has frequently found its way to the open market. It is to be noted, however, that this inferior condition has been largely due to natural causes and has consisted more of variations in the genuine bark rather than in its adulteration by other forms. The influences which brought about these variations do not seem to have been active during the past few years or if so their effects have not been mentioned. Critical examinations of numerous commercial shipments of the drug have served to confirm the former supposition. The entire contents of each bag of drug composing these shipments was inspected and found to reveal no greater variations than those which naturally occur in all drugs of vegetable origin. It has been learned that in the inspection of all such materials certain limits, to all natural variations, must be fixed in order to accomodate the constant and unavoidable fluctuations. During the past few years the maintenance of a standard with rather strict limits has been found possible, in the case of cascara bark, owing to the fact that the drug has been of such uniform character. Nevertheless, such a condition of uniformity never continues indefinitely. Interruptions and indeed actual reversals in the market situations may occur at the least expected moment. An occurrence of this nature or any interruption of a commercial aspect, relative to the cascara supply would seem of sufficient importance to justify its announcement. It is, therefore, with this view in mind that the present discussion has been suggested.

The appearance of a new form of inferior bark has been detected during a routine inspection of a considerable lot of cascara sagrada. Though not having been extremely troublesome it presents possi-

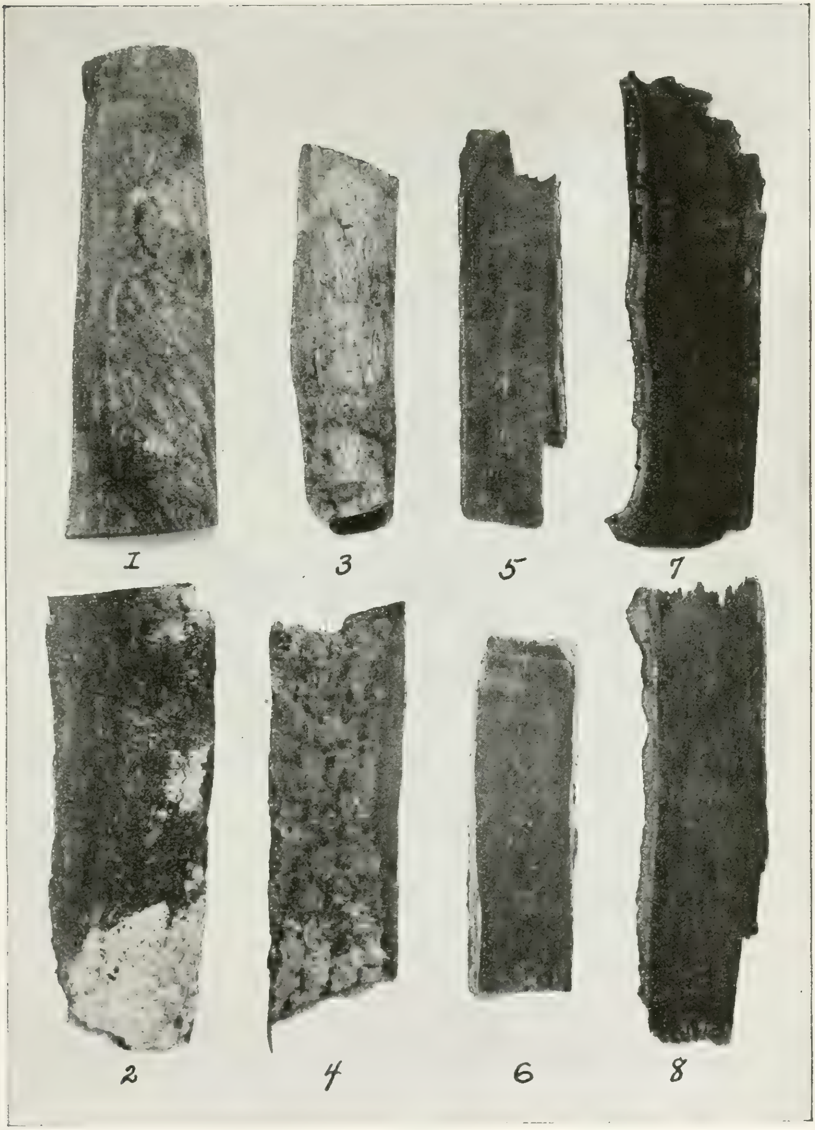

PLATE I.
Fig. 1, 3, 5 and 7, Cascara sagrada. Fig. 2, 4, 6 and 8, Cascara substitute.

bilities which if taken advantage of by the collectors would make it a difficult form to control. The bark in question resembles genuine cascara quite closely and especially is this true of the periderm. See fig. 1, 2, 3 and 4, plate I. Figures 1 and 2 are almost indistinguishable while in 3 and 4 is brought out the principle exterior difference viz., the more prominent longitudinally elongated lenticles of the substitute (fig. 4) and the small inconspicuous transversely elongated ones of the cascara (fig. 3). The inner surface, however, and especially of the large pieces is the key to the ready detection of the adulterant. This difference being largely one of color is not indicated to advantage in the photograph. This color in the false bark is a light brown with frequent darker blotches. None of the yellow or lustrous brown of the true bark is evident. The inner surface and fracture are also diagnostic, being in the spurious article, respectively striate and fibrous, while in the cascara they are smooth and short. The bark is practically odorless and the taste is only that of astringency with slight bitterness. The size and form of the adulterant cannot be regarded as important characters since

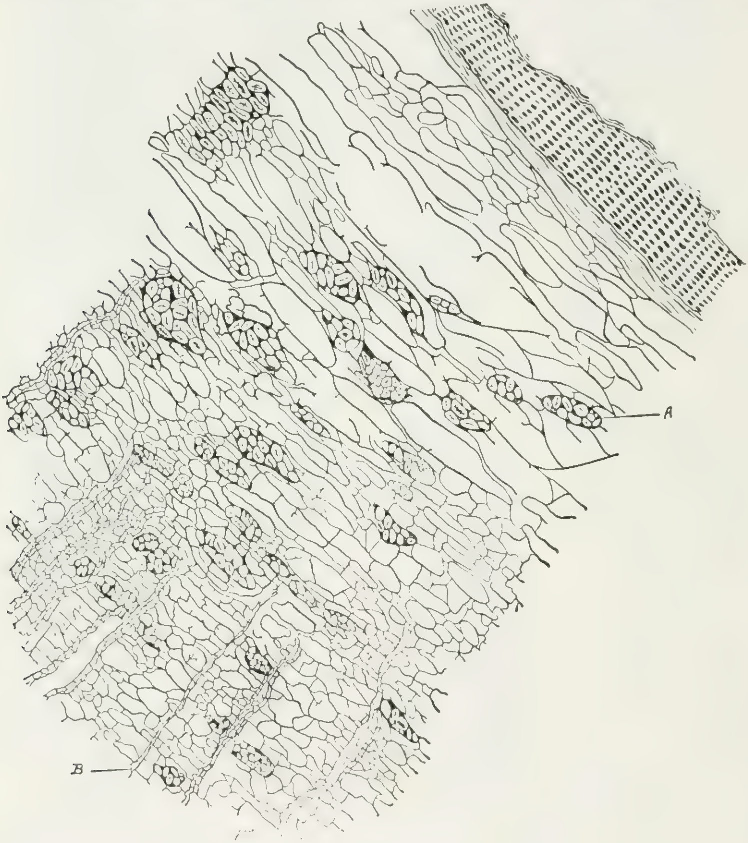

PLATE II.
Transverse section, Cascara sagrada bark.
A—Sclerotic cells. B—Medullary ray.

they both exhibit about the same range of variations as the genuine bark. The adulterant is somewhat flatter, showing no tendency to form quills, and is slightly thicker.

Turning to the microscopic structure, this is found to disclose an even greater difference than the external features. (See plates II and III). These plates were outlined with a camera lucida attachment under a magnification of 87 diameters and the detail filled in under a magnification of 385 diameters. It will be noted from these plates that the extreme width and conspicuous nature of the medullary rays, the difference in the bast fibers and the absence of sclerotic cells are the principle features by which the adulterant may be distinguished from the true bark. A consideration of all histological elements is unnecessary and of no practical value in this

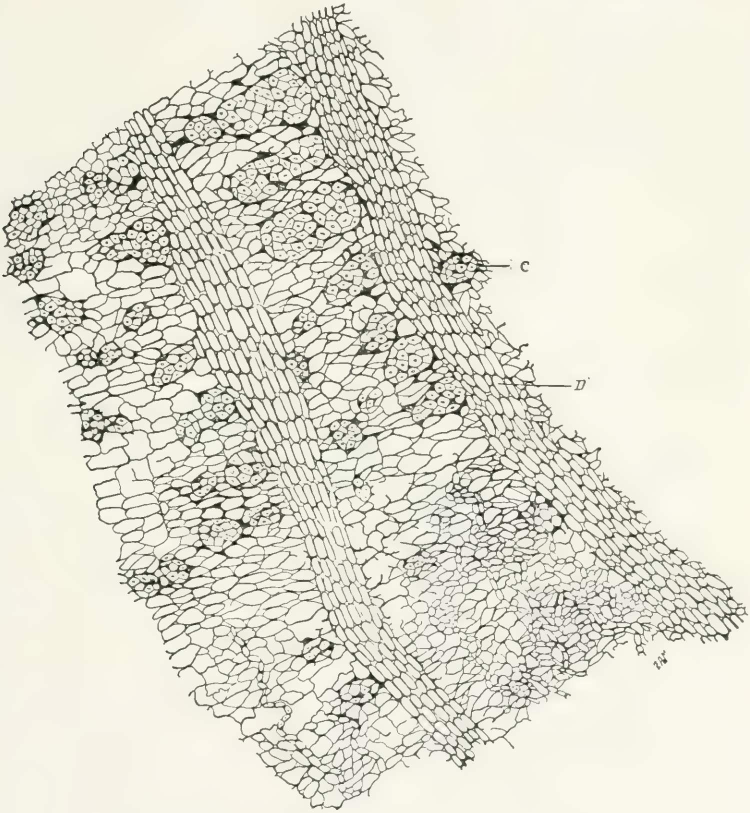

PLATE III.
Transverse section of bark, Cascara substitute.
C—Bast fibers, D—Medullary ray.

particular case. The cortical portions of the bark have not been included in Plate III on account of the absence of any important structures. The outer and inner layers of this area are to all practical purposes the same in both cases and could be disregarded in routine inspection.

Although the form herein described has not been identified it is seen to possess none of the taxonomic characters of the genus Rhamnus and so can be eliminated from this group of plants. Of greater importance, however. than its identity is its isolation from the true bark. To accomplish this, all commercial packages of the crude drug should receive a careful botanical inspection. This inspection should not only cover all commercial packages but also every portion of these packages. This is best carried out during the process of manufacture when all packages must of necessity be broken and the drug handled in the loose form.

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# A STUDY OF AMERICAN GROWN CANNABIS IN COMPARISON WITH SAMPLES FROM VARIOUS OTHER SOURCES.

C. R. ECKLER AND F. A. MILLER.

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Several factors have recently given rise to considerable comment on the subject of American Cannabis. Of these, perhaps the most important are: the increased cost of the Indian drug, resulting in the search for a cheaper product; the none too well supported claims made by some investigators, leading, we believe, to a false conception of the activity of commercial lots of drug; the question as to whether or not an active variety can be successfully cultivated in this country on a commercial scale; and the question of the feasibility of including the American variety in the coming revision of the United States Pharmacopœia. The influence upon the activity of the drug of such factors as soil, climate, geographical location, time of harvesting, method of curing, and parts of plant included, are also of interest.

Famulener and Lyons[1] claim that the character of the soil and the geograhical area where grown, primarily influence the activity of the plant. Holmes[2] reported on French, African and Indian Cannabis in 1905 to the effect that although the French and African varieties indicated some activity, they were not nearly so active as the Indian drug and could not be recommended for manufacturing purposes. He reported the African as being a little more active than the French. Houghton and Hamilton,[3] in 1908, named seven different sources from which they had tested samples, three being within the United States. In their conclusions they claim that Cannabis sativa, when grown in various localities of the United States, is found to be fully as active as the best imported Indian grown Cannabis. True and Klugh[4] grew plants from foreign seed and commercial drug, at Washington and southern Texas. They reported that the home grown drug was found to be fully equal in efficiency to the imported article. Hamilton[5] called attention to the fact that "various investigators," whom he failed to name, have examined American hemp and obtained results which indicated that the influence of soil and climate does not effect the quality of the extract.

Our interest in the different phases of the Cannabis situation has led us to report our experience with various commercial samples of the drug, and to state in brief the results of our work on the experimental cultivation of the American and Indian varieties. Materials for these investigations were obtained by growing plots, experimentally, both from the foreign and native seed. The following tests were carried out on various samples collected from these plots.

CULTIVATED CANNABIS.

| Where grown | No .of sample | Percentage activity compared with good Indian grown samples. |
|---|---|---|
| Plot A | B-176 | Approximately 40% |
| Plot A | 1494 | Approximately 50% |
| Plot B | B-596 | Approximately 60% |
| Plot C | B-770 | Not more than 50% |
| Plot C | B-771 | Not more than 50% |
| | B-703 | Approximately 65% |
| Plot D | B-177 | Approximately 40% |
| Plot E | 1493 | Approximately 40% |
| | B-437 | Approximately 50% |
| | B-693 | Not more than 50% |

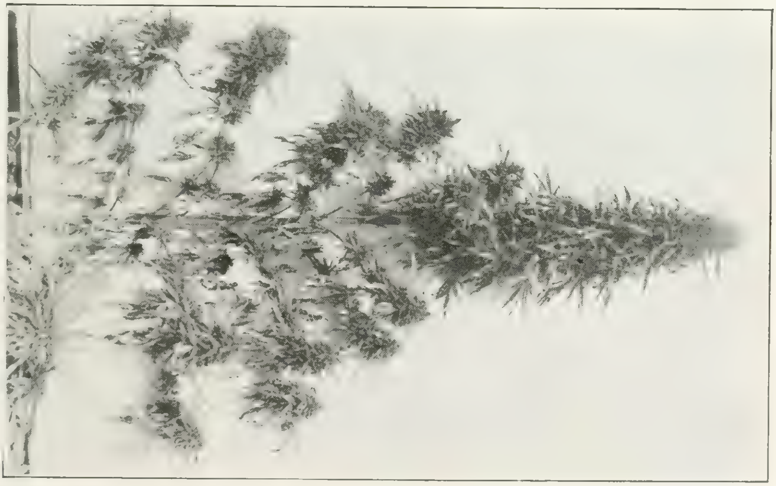
The samples on which the foregoing assays were made, were obtained under the following conditions: Seeds from an active commercial lot of Indian Cannabis were taken for experimental planting. A good stand of plants was easily obtained from these seeds. The plants were grown under ordinary agricultural condiditions, upon a soil consisting of rather poor clay loam. The plants as observed on a plot (A) thirty by sixty feet, exhibited a wide range of variations. These variations were indicated by such botanical characteristics as size, color, and form, as found in leaf, plant, and inflorescence, date of flowering and time of maturity. Twelve plants from this plot (A) were of dwarf habit, from one to two feet high, and free from branches. These plants flowered early. They produced pistillate flower clusters which were heavy, compact, and leafless, averaging from two to four inches in length. They bore much resin and possessed the characteristic odor of Indian Cannabis. The three earliest flowering of these were selected for seed plants. August 17th, 1909, the pistillate inflorescence of the twelve dwarfs was collected for testing. The sample was cured at room temperature and designated by No. B-176. The remainder of the plants in plot A were from three to seven feet high, much branched, and produced small inconspicuous flower clusters which were late in appearing. They showed but few resin bearing glands and did not possess the odor of foreign Cannabis to such a marked degree as noticed in the dwarf plants.

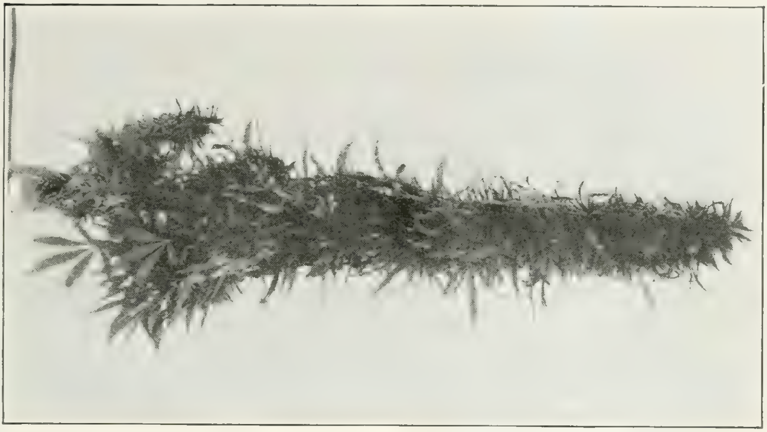
August 24th, 1909, the pistillate tops, including several inches of leafy stem, were collected for testing. The sample thus obtained was dried at room temperature, and designated by No. 1494.

The seeds selected from the three early flowering dwarfs were planted the following year on plot B. The plants obtained were a great improvement over the parents of the previous year. They averaged from two to five feet in height and were more branched. The inflorescence was long (averaging over twelve inches), dense, heavy, and carried much resin. Individual flower clusters were observed, measuring fourteen inches in length and bearing no leaves except the small bracts subtending the flower clusters. September 7th, 1910, a collection was made from twelve of the best plants. The sample thus obtained was cured at room temperature, and tested under No. B-596.

At the same time, seed plants were selected. These selections were made for the purpose of continuing and improving the strain as follows: first, from one plant bearing the largest inflorescence, which in this instance measured fourteen inches in length; second, from one plant showing a deep purple color in all parts; and third; from twelve of the best remaining plants, the size of the inflorescence serving as a basis for the selection. The following year the seeds from these selections were planted on plot C. The resulting crop consisted of uniform plants, very much like the parents in most cases. One marked exception was the appearance of the purple color in many plants from each of the other selections. It predominated, however, in the second selection. The first selection resulted in low, almost unbranched individuals. The pistillate flowering tops averaged ten inches in length, and produced more leaves than the parent form. A collection was made from these plants, for testing, on October 13th, 1911. This sample was taken from the twelve best plants and tested under No. B-770. The second selection gave rise to individuals, all but a small proportion of which possessed the purple characteristic to a marked degree. The pistillate tops averaged one foot in length but were of a more interrupted nature and not so dense as noted in the parent plants. A collection was made from these plants October 13th, 1911, and designated by No. B-771. No collection was made from the third selection. From sample B-770 three individual plant selections have again been made; first from one plant four feet high, and unbranched, which produced a large, single leafless inflorescence; second, from one plant three feet high, unbranched, which produced a large, single leafy inflorescence; and the third from one plant four feet

American Grown Cannabis from Foreign Seed.





Individual plant selections of Cannabis from B-771, showing distinct differences in type of plant and character of inflorescence. The entire plants are shown in the photographs.

high, which was divided near the top into several short branches. All of these produced good flower clusters, with numerous leaves. The strain showing the purple character was discontinued.

Sample B-703 was grown from seeds taken from a commercial lot of Cannabis Indica, No. 1728. These plants were unusual as to size and vigor, but produced an extremely low percentage of flowering tops.

Samples No. B-177 and 1493 were grown from seeds of American hemp, obtained in Lexington, Ky. The location of the plots (D and E) and the conditions of growth were practically the same as those for the foreign seed. The tops of this variety were very poor, being small and bearing a large proportion of leaves. August 17th, 1909, a sample was collected which consisted largely of leaves and stems. This was dried at room temperature and tested under No. B-177. A second sample for testing (1493) was collected, August 24th, 1909. The drug was spread thinly in the field for twenty-four hours, during twelve of which it was exposed to direct sunlight. Drying was then completed under shelter. The following year another planting was made from the Kentucky seed under practically the same conditions. The character of the plants was the same as noted for the preceding year. A sample of the leafy tops was tested under No. B-437. No further investigation has been made of this form. The pistillate inflorescence was in all cases very small, leafy, and lacking in resin and the true Cannabis odor.

Sample No. B-693 was obtained from one extremely large and luxuriant plant. This plant was observed in a hardy border, and was tested on account of its extreme size and the very favorable appearance of its inflorescence. The exact source of the seed is not known.

In order to gain some information regarding the activity of commercial lots of American Cannabis a number of samples were purchased from drug brokers and tested physiologically. The method in brief is as follows:

### METHOD OF PREPARING DOSES FROM CRUDE DRUGS.

A 20 gramme sample of the drug in No. 60 powder is macerated with official alcohol in a small flask, which is occasionally agitated, for from 48 to 72 hours. The content of the flask is then poured into a narrow percolator. Percolation is allowed to proceed slowly, more alcohol being added as necessary. The first 90 cc. of percolate are reserved. 100 to 150 cc. more are then collected, evaporated under an air jet without heat, added to the reserved 90 cc. and made up to 100 cc. Doses of this 20% tinture are calculated, for each dog, per Kgm of body weight. The quantity is drawn off with a pipette, evaporated without heat to an extract consistency, and made into a pill or put into a capsule.

In order to determine with certainty whether or not the active principles were completely extracted by the foregoing method, on several occasions the drug was returned to the flask and again macerated and percolated. 100 cc. of percolate were reduced, made into a pill, and given to a small susceptible dog. In no case were noticeable symptoms produced.

## METHOD OF TESTING.

The work is carried out on fox terriers which are kept in well ventilated, comfortable stalls. These stalls are arranged in a row, and so constructed that the dogs cannot see each other, or any object about the room which might disturb or excite them. The assays are made by one person, usually, who endeavors to maintain a thorough acquaintance with the normal movements of each animal. When a preparation is to be tested one, two or three pairs of dogs are selected which have previously been standardized against Cannabis preparations. The animals are fasted for twenty-four hours, and then to one of a pair is given (by mouth, in pill or capsule) a dose of a standard preparation, and to the other a dose of the unknown. The animals are observed almost constantly during the period between one and three hours after the administration and the results noted. After this, the animals are allowed sufficient time for recovery and complete excretion of the drug, usually about three days, and then the drugs are given again, this time in the reverse order. The next time the order is again reversed and so on. The doses are increased or diminished according to the effects previously produced, until the minimum amount of each preparation necessary to produce slight but distinct inco-ordination of the muscles is determined.

The dose of the unknown required for a given animal is compared with that required of the standard. The results on the several animals are then compared and conclusions drawn.

AMERICAN CANNABIS FROM VARIOUS COMMERCIAL SOURCES.

| No. of sample | Extractive | Percentage activity compared with good Indian grown samples | Commercial Source |
|---|---|---|---|
| 1032 | | Not more than 20% | Indianapolis |
| B-407 | | Not more than 50% | St. Louis |
| B-529 | | Not more than 40% | New York |
| B-824 | | Not more than 65% | Indianapolis |
| B-1034 | 7.98% | Not more than 50% | St. Louis |
| B-1040 | 11.08% | Not more than 50% | New York |
| B-1039 | 11.39% | Not more than 75% | New York |
| B-1047 | 13.60% | Not more than 60% | St. Louis |
| B-1054 | 15.47% | Not more than 75% | New York |

The following conditions were noted in the foregoing samples:

No. B-529 contained thirty per cent. of cut stems. The proportion of pistillate inflorescence was small. The odor was not pronounced or characteristic of Indian Cannabis.

No. B-1034 contained an excess of leaves, stems and seeds. The flowering tops were small.

No. B-1039 contained over fifty per cent of seeds and stems. The color of the sample was a uniform bright green. No odor of Cannabis was suggested.

No. B-1040 contained only a small proportion of seeds and stems. The drug had been compressed in such a manner as to resemble Indian Cannabis.

No. B-1047 contained a very small proportion of leaves, stems and seeds. The odor was slight and not that of Indian Cannabis.

No. B-1054 consisted of small leaves, leaf fragments, and bracts. No seeds or stems were present.

No. B-824 and No. 1032 were collected from wild plants of Cannabis sativa. and consisted largely of leafy tops. The flower clusters were extremely small, and constituted only a minor portion of the samples.

## INFERIOR CANNABIS FROM FOREIGN SOURCES.

That there is much inferior so-called Indian Cannabis on the market is true without doubt. It seems probable that a great deal of this has been grown, not in India, but in other places.

The following table shows the results obtained in testing some of these inferior grades. These samples were received at different times (over a period of two years), and were submitted by drug brokers upon the requests for samples of Indian Cannabis.

| No. of Samples | Marks on Package | Activity compared with good Indian samples | Commercial Source |
|---|---|---|---|
| B-275 | "Cannabis Indica" | Approximately 70% | Greece |
| B-634 | "Indian Cannabis"<br>"Green Tops" | Approximately 60% | Germany |
| B-644 | "Cannabis Herb, Madagascar." | Approximately 50% | Germany |
| B-660 | "Cannabis Indica Herb." | Approximately 40% | Greece |
| B-815 | "Levant." | Approximately 50% | New York |
| B-812 | "East Indian Guaza" | Approximately 90% | London |

## COMMERCIAL FLUID EXTRACTS OF AMERICAN CANNABIS

Since the crude American Cannabis upon the market proved to be generally low and variable in activity, it was of interest to know whether or not commercial fluid extracts would show these same qualities. Several samples were purchased in the market and the results of these tests may be seen in the following table:

| No. of Sample | Amount of extractive | Activity compared with same makers' fluid extracts from Indian grown drug |
|---|---|---|
| P-307 | 7.40% | Approximately 90% |
| P-308 | 9.86% | Approximately 100% |
| P-309 | 6.16% | Not more than 75% |
| P-310 | 6.37% | Not more than 50% |
| P-311 (African) | 15.50% | Between 90%-100% |

## SUMMARY.

Soil, climate and geographical location have a decided influence upon the activity of American and Indian Cannabis.

Repeated plantings from carefully selected seeds of American and Indian Cannabis have failed to yield a product testing over 65% as active as good Indian grown drug, while the majority of the plantings tested 50% and less.

Commercial samples of American Cannabis were found to vary widely in their activity. Of the samples tested none were as active as good samples of the Indian drug, and a number were not more than 50% as active.

Commercial samples from various foreign sources were supplied upon requests for samples of Cannabis Indica. None of these are equal to the Iudian drug and some tested extremety low.

Commercial samples of fluid extracts of American Cannabis vary widely in their activity, some being not more than fifty per cent as active as Indian fluid extracts from the same makers.

In addition to physical and botanical characteristics, the physiological assay is of greatest importance in judging the quality of the drug. Very little dependence can be placed on the estimation of the extractive matter yielded to alcohol.

The results of this work indicate that if American Cannabis is made official, difficulty will generally be experienced in obtaining highly active lots which will compare favorably with good Indian drug.

[1] Famulcner & Lyons; Proc. Am. Pharm. Assoc., 1903, 51, 240.

[2] Holmes: Pharm. Jr,, 1905, 74, 550.

[3] Houghton & Hamilton: Am. Jr. Pharm., 1908, 80, 21.

[4] True & Klugh: Proc. Am. Pharm. Assoc., 1909, 57, 843.

[5] Hamilton: Jr. Am. Pharm. Assoc., 1912, 1, 201.

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# THE BROMINE AND IODOMETRIC METHODS FOR THE VOLUMETRIC DETERMINATION OF CRESOL.

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Of the several methods suggested for the volumetric determination of phenol, the bromine method has received the most consideration, and in almost every instance where bromine has been used an attempt has been made to substitute iodine in its stead.

The popularity of the bromine method is no doubt due to two causes: First, to the fact that it answers the demand for a reliable method in the determination of the first member of the series, namely, phenol; second, to the ease in manipulation resulting from the use of the Koppeschaar bromine solution.

Now cresol is the next member of the homologous series; however, it presents, upon treatment with bromine solution and acid, problems quite different from that of phenol. Since commercial cresol is a mixture of the three isomeric phenols *o-*, *m-* and *p*-cresol, the presence of the $CH^3$ group in the different positions necessitates the formation of compounds structurally unlike and empirically the same, and also causes, in the case of meta cresol, the formation of compounds both structurally and empirically different from those formed by *o-* and *p*-cresols. In 2, 4, 6-tribom phenol brom, the bromine occupies both the *o-* and *p*-positions. Now in the case of *o-* and *p*-cresol the $CH^3$ group occupies the *o-* and *p*-positions and only dibromcresol and dibromcresol brom compounds can be readily formed since the $CH^3$ group is more difficult to replace by free Br than is a hydrogen atom. However, the $CH^3$ group is slowly replaced and prolonged standing (several days) results in formation of tribromphenol brom—W. Autenrieth and F. Beuttel[2] have recently published the results of an extensive investigation along this line.

Meta cresol forms tribrom-*m*-cresol brom since the $CH^3$ group is not in the position that the Br atom would occupy.

H. Ditz and F. Cedivoda[3] have proposed a bromine method for cresols depending upon the difference in speed of reactions between bromine and *m*-cresol on the one hand and bromine and *o-* and *p*-cresols on the other. If either *o-* or *p*-cresol is mixed with a known excess of bromine solution, acidfied with (1 : 1) hydrochloric acid and agitated for 1 min., on adding KI and titrating with thiosulfate it will be found that each molecule of *o-* and *p*-cresols has absorbed 2 atoms of bromine. Under the same conditions each molecule of *m*-cresol will absorb 3 atoms of bromine. Now if acidification is effected by concentrated sulfuric acid, the solution shaken for five minutes and insoluble matter removed by filtration, 3 atoms of bromine will have combined with each molecule of *o*- and *p*-cresol, 4 atoms with each molecule of *m*-cresol.

The above method depends for its success upon definitely fixed conditions; namely: time, strength of acid, excess of bromine, etc. F. Russig and G. Fortmann[4] have criticized this method and H. Ditz[5] has replied at length.

The present study of the reaction of bromine and iodine upon the different cresols was made for the purpose of finding which of the cresols were amenable to assay by processes which do not require previous knowledge concerning the percentage of product, that is, by processes dependent upon the formation of definitely fixed, comparatively stable, final products; namely: compounds containing three atoms of bromine in combination.

Samples of the individual cresols were redistilled and volumetric solutions prepared from the distillates, small amounts of N/2 NaOH being added to facilitate solution. Aliquot portions of these solutions were used in the several determinations.

The results appearing in the tables are based upon the formation of dibromcresol brom and tribrom compounds on the one hand and diiodo compounds on the other.

Ortho cresol does not readily form a stable dibrom-*o*-cresol brom or a tribrom compound as is evidenced in Exp. I. However, upon 1 1/2 hours' standing more than sufficient Br is absorbed for the

ORTHO CRESOL

| No. | Cc. Ortho cresol | Cc. N/10 Br | Cc. $H^2O$ | Cc. Conc HCl | Time of standing | Cc. 20% KI. | Cc. Conc. HCl | Time of standing Hrs. | Results Per cent |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 20 | 50 | 175 | 5 | 20 minutes | 10 | — | 1 1-2 | 94.45 |
| 2 | 20 | 50 | 175 | 5 | 1 1-2 hours | 10 | — | 1 1-2 | 103.5 |
| 3 | 20 | 50 | 175 | 5 | 1 1-2 hours | 10 | — | 2 1-2 | 102.7 |
| 4 | 20 | 50 | 175 | 5 | 1 1-2 hours | 30 | — | 3 1-2 | 101.1 |
| 5 | 20 | 50 | 275 | 5 | 1 1-2 hours | 20 | — | 2 | 100.98 |
| 6 | 20 | 50 | 275 | 5 | 1 1-2 hours | 206 | — | 2 | 100.68 |
| 7 | 20 | 50 | 275 | 5 | 1 1-2 hours | 20 | — | 14 | 99.78 |
| 8 | 20 | 50 | 275 | 5 | 1 1-2 hours | 30 | — | 2 | 99.98 |
| 9 | 20 | 50 | 275 | 5 | 1 1-2 hours | 30 | — | 3 1-2 | 100.17 |
| 10 | 20 | 50 | 275 | 5 | 1 1-2 hours | 30 | — | 4 1-2 | 99.95 |
| 11 | 20 | 75 | 275 | 5 | 1 1-2 hours | 20 | — | 2 | 109.4 |
| 12 | 15 | 50 | 275 | 5 | 1 1-2 hours | 30 | — | 18 | 105.15 |
| 13 | 10 | 50 | 275 | 5 | 1 1-2 hours | 30 | — | 18 | 106.85 |
| 14 | 15 | 50 | 275 | 5 | 3 hours | 30 | — | 5 min. | 113.3 |
| 15 | 15 | 50 | 275 | 2.5 | 5 hours | 30 | — | 18 | 106.9 |
| 16 | 10 | 50 | 275 | 2.5 | 4 hours | 30 | 5 | 20 | 93.4 |
| 17 | 15 | 50 | 275 | 2.5 | 5 hours | 30 | 5 | 40 | 86.22 |

| Number | cc Ortho cresol | cc. N/10 I. | g. CH$^3$COONa 3H$^2$O. | Time of standing. | Procedure. | Result. Per cent. | Excess N-1 0 I, cc. |
|---|---|---|---|---|---|---|---|
| 18 | 25 | 50 | 5 | 30 minutes | Diluted, added 5 cc CHC$^2$ titrated with N-10 thiosulphate, using starch as indicator. | 93.66 | ±25 |
| 19 | 25 | 50 | 5 | 1 1-5 hrs. | | 100.04 | ±25 |
| 20 | 25 | 50 | 5 | 14 hours | | 100.71 | ±25 |
| 21 | 25 | 50 | 9 | 1 hour. | | 99.93 | ±25 |
| 22 | 25 | 50 | 9 | 2 hours. | | 100.1 | ±25 |

formation of these compounds and the addition of 10 to 30 cc. of 10 per cent. KI with from 1 1/2 to 3 1/2 hours' standing does not result in the liberation of the bromine necessary for the formation of either dibrom-*o*-cresol or a tribrom compound as would occur if reaction proceeded in a manner strictly analogous to phenol (Exps. 2, 3 and 4.)

Now a greater dilution results in the absorption of a smaller quantity of bromine but results are still too high, (Exps. 5 and 6.) Increased standing after addition of 20 cc. 20 per cent. KI causes a reversal with practically theoretical quantities of bromine absorbed, the time of standing being materially reduced upon increase in the quantity of KI (Exps. 7, 8, 9 and 10).

An increase in concentration of bromine in contact with *o*-cresol as is effected by larger quantities of Br solution or a decrease in the quantity of cresol solution results in the absorption of much more than the theoretical quantity of bromine and even upon prolonged standing in contact with increased quantities of KI, suitable reversal does not take place (Exps. 11, 12, 13, 14 and 15.)

The effect due to increase in the quantity of acid is very marked. When bromine is liberated by one-half the usual quantity of concentrated HCl sufficient reversal does not take place after addition of KI (Exps. 14 and 15). However, when an additional 5 cc. of concentrated HCl are added at the same time as the KI, the reversal causes far less than theoretical quantities of bromine to remain in combination (Exps. 16 and 17).

Thus it will be seen that practical results dependent upon the formation of a dibromcresol brom or tribrom compound can not be obtained except by definitely fixing the several conditions of the experiment in pursuance of which a previous knowledge of the percentage of *o*-cresol is required.

Now when iodine acts upon *o*-cresol, diiodo-*o*-cresol is formed. The reaction is reversible and sodium acetate is added to unite with the hydriodic acid produced by the substituting iodine, thereby causing the reaction to proceed to completeness (Exps. 18, 19, 20, 21 and 22). In this case the reaction ends with the formation of the diiodo compound, hence it is made the basis of the following method for the determination of *o*-cresol.

Dissolve 2-2.5 g. *o*-cresol in distilled water containing about 10 cc. N 2 NaOH to facilitate solution. Dilute to 1000 cc. Withdraw 25 cc. portions and place in a 500 cc. glass stoppered receptacle. Add to the receptacle approximately 9 grams sodium acetate and then 50 cc. N/10 I solution. Let stand 1 hour, dilute with 100-200 cc. distilled water, add a few cc. of CHCl$^3$ to dissolve the precipitated diiodocresol and titrate with N-10 thiosulphate, using starch as an indicator.

1 cc. N/10 I = 0.002681 g- *o*-cresol.

Meta cresol does not rapidly form tribrom-*m*-cresol brom with the subsequent formation of tribrom-*m*-cresol upon standing in contact with KI (Exps. 1, 2 and 3). If the *m*-cresol solution stands 1 1 2 hours in contact with the bromine and then 10 cc. 20 per cent. KI are added and solution is allowed to stand 1 to 2 hours, all the cresol is converted into tribrom-*m*-cresol (Exp. 4).

Dilution permits a more rapid reaction which almost completes itself in 1 2 hour. The addition of KI causes a complete reversal even though the time of standing in contact with free bromine has been much prolonged (Exps. 5. 6, 7, 8, 9, 10 and 11).

META CRESOL.

| No. | Cc. Meta cresol | Cc. N/10 Br. | Cc. H$^2$O | Cc. Conc. HCl. | Time of standing | Cc. 20 % KI. | Cc. Conc. HCl. | Time of standing | Result |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 20 | 50 | — | 5 | 1 min. | 10 | — | 1 hour | 97.9 |
| 2 | 20 | 50 | — | 5 | 1-2 hr. | 10 | — | 2 hours | 98.48 |
| 3 | 20 | 50 | — | 5 | 1 hour | 10 | — | 1 hour | 99.2 |
| 4 | 20 | 50 | — | 5 | 1 1-2 hrs | 10 | — | 2 hours | 99.9 |
| 5 | 20 | 50 | 175 | 5 | 1-2 hour | 10 | — | 2 hours | 99.6 |
| 6 | 20 | 50 | 175 | 5 | 1 1-2 hrs | 10 | — | 2 hours | 100.07 |
| 7 | 20 | 50 | 175 | 5 | 3 hours | 10 | — | 18 hours | 99.93 |
| 8 | 20 | 50 | 175 | 5 | 6 hours | 40 | — | 18 hours | 99.81 |
| 9 | 15 | 50 | 175 | 5 | 6 hours | 10 | — | 5 min. | 101.11 |
| 10 | 15 | 50 | 175 | 5 | 6 hours | 10 | — | 1-2 hour | 100.5 |
| 11 | 15 | 50 | 175 | 5 | 6 hours | 10 | — | 1 1-2 hr. | 100.2 |

| No. | cc. Meta cresol | cc. N/10 I. | g. $CH_3COONa,3H_2O$ | g. $NaHCO_3$ | Time of standing | Procedure. | Result. Per cent |
|---|---|---|---|---|---|---|---|
| 12 | 25 | 40 | 9 | — | 1 hr | Diluted, added about 5 cc. $CHCl_3$ and titrated with N 10 thiosulphate, using starch indicator. | 118.5 |
| 13 | 25 | 40 | - | 0.5 | 1 hr | | 122.0 |
| 14 | 25 | 40 | - | 2.0 | 1 hr | | 130.0 |
| 15 | 25 | 50 | - | 2.0 | 2 hrs. | | 155.0 |
| 16 | 25 | 50 | 9 | — | 2 hrs. | | 152.0 |

It is thus apparent that *m*-cresol may be determined by a conversion into the tribrom compound.

Upon the other hand, when iodine[7] acts upon *m*-cresol in the presence of sodium acetate or sodium bicarbonate, a diiodo compound that may be made the basis of calculation is not produced. More than the theoretical quantity of iodine is absorbed (Exps. 12, 13, 14, 15 and 16).

PARA CRESOL

| No. | Cc. Para cresol | Cc. N/10 Br | Cc. $H_2O$. | Cc. Conc. HCl. | Time of standing | Cc. 20 % KI. | Cc. Conc. HCl. | Time of standing | Result Per cent. |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 20 | 50 | 275 | 5 | 1 hr. | 10 | — | 2 hrs. | 81.1 |
| 2 | 20 | 50 | 275 | 5 | 4 1-2 hrs. | 10 | — | 18 hrs. | 95.73 |
| 3 | 20 | 50 | — | 5 | 4 1-2 hrs. | 10 | — | 18 hrs. | 76.35 |
| 4 | 15 | 50 | 275 | 2.5 | 3 hrs. | 10 | — | 5 min. | 97.34 |
| 5 | 15 | 50 | 275 | 5 | 1 1-2 hrs. | 30 | — | 18 hrs. | 89.73 |
| 6 | 10 | 25 | 200 | 2.5 | 3 hrs. | 10 | — | 18 hrs. | 101.8 |
| 7 | 10 | 25 | 200 | 2.5 | 3 hrs. | 20 | — | 18 hrs. | 101.75 |
| 8 | 10 | 25 | 200 | 5 | 3 hrs. | 10 | — | 18 hrs. | 97.7 |
| 9 | 10 | 25 | 200 | 2.5 | 8 1-2hrs. | 10 | — | 16 hrs. | 104.6 |
| 10 | 10 | 25 | 200 | 2.5 | 4 hrs. | 30 | 5 | 20 hrs. | 84.94 |
| 11 | 10 | 25 | 200 | 2.5 | 5 hrs. | 30 | 5 | 40 hrs. | 82.90 |

| No. | cc. Para cresol. | cc. N/10 I. | g. $CH_3COO$ Na,3 $H_2O$. | Time of standing | Procedure. | Result. | Excess. cc. |
|---|---|---|---|---|---|---|---|
| 12 | 25 | 30 | 9 | 1 hr. | Diluted, added 5 cc. $CHCl_3$ titrated with N/10 thiosulphate, using starch an indicator. | 98.81 | 25 |
| 13 | 25 | 30 | 15 | 1 hr. | | 99.56 | 25 |
| 14 | 25 | 40 | 9 | 1 hr. | | 99.75 | 25 |
| 15 | 25 | 50 | 9 | 1 hr. | | 99.75 | 25 |
| 16 | 25 | 50 | 0 | 2 hrs. | | 99.75 | 25 |

Para cresol absorbs bromine much more slowly than either of the other cresols. Under similar experimental conditions, 4 1/2 hours' standing in contact with free bromine results in absorption of only 95.73 per cent. of the theoretical quantity (Exps. 1, 2 and 3). If concentration of free bromine is increased and usual quantity of HCl decreased one-half, the bromine in combination exceeds the amount necessary for the formation of a dibrom-*p*-cresol brom or tribromcresol compound and 18 hours' standing does not result in sufficient decomposition even though the quantity of KI is doubled (Exps. 4, 5, 6, 7, 8 and 9).

However, an increase in both HCl and KI results in a most pronounced reversal[8] which is due to the decomposition of dibrom-*p*-cresol brom into dibrom-*p*-cresol (Exps. 10 and 11),

Here again the result is directly dependent upon conditions and previous knowledge is necessary on part of the operator.

Now when iodine reacts with *p*-cresol in presence of the condensing agent, sodium acetate, diiodo-*p*-cresol is formed in theoretical quantity if sufficient time is allowed for reaction to complete itself.

This reaction is made the basis of a method for the determination of *p*-cresol (Exps. 12, 13, 14, 15 and 16). The precedure is exactly the same as that given under *o*-cresol.

## SUMMARY

1. Usual bromine methods for the determination of cresol depend upon fixed conditions presupposing a previous knowledge on the part of the operator and while they may be of service they do not satisfy the demand for a desirable method.
2. Tribrom-*o*- and *p*-cresol brom compounds can not be formed in a manner analogous to the production of 2, 4, 6-tribrom phenol brom. Dibromcresol brom compounds are formed and finally tribromphenol brom; however, *o*- and *p*-cresols can not be determined volumetrically by a conversion into these compounds.
3. Meta cresol is determined by a conversion into tribrom-*m*-cresol.
4. Ortho and para cresols form diiodo compounds, and this reaction is made the basis of a method for their determination.
5. Meta cresol does not yield diiodo compounds under the same conditions; hence, cresol U. S. P. or any mixture containing *m*-cresol can not be determined by a volumetric method.

*Chemical Research Department.*

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2. "Ueber die Bestimmung des Phenols, Salicyl alkohols, der Salicylsäure und p-oxybenzoësäure als Tribrom phenol brom" (Autenrieth und Beuttel), Archiv der Pharm., 1910, 112.
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6. Heated to 40° C. after addition of KI.
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# THE ALKALOIDAL CONTENT OF INDIVIDUAL PLANTS OF DATURA STRAMONIUM L. AND DATURA TATULA L.

F. A. MILLER AND J. W. MEADER

Reprinted from original communications, Eighth International Congress of Applied Chemistry.
Vol. XVI—Page 57.

The examination of individual plants of *Datura Stramonium* L. and *Datura tatula* L. for their total alkaloidal content has been undertaken for two reasons; first, as a means of following the effects of prolonged cultivation upon the percentage of alkaloids, and second as a means of selecting high yielding individual plants. These high yielding plants are intended to serve as parents of future generations from which continued selections can be made. In these selections the object is to develop pure strains, which it is hoped, will exhibit a racial improvement in relative alkaloidal value over wild plants or those cultivated without any effort toward improvement. It has been shown[1] that the application of certain nitrogenous fertilizers to members of the solonaceous family and especially belladonna, will cause a perceptible increase in the alkaloidal content. This increase, however, does not represent an improvement as understood by plant breeders. It is environmental and thus only temporary. What is most needed to further the advancement of drug growing is a permanent improvement of the more valuable medicinal plants. This may be accomplished through the application of special methods of breeding as now practiced by plant breeders, horticulturists and florists.

Little has been done on the cultivation and improvement of stramonium except in the case of a few species, which are grown for decorative purposes. Experimental plots of several species have been grown, but apparently have not been continuously cultivated. As to improvement, there is no evidence of any attempts having been made, except that by Meyer[2] to hybridize Datura Metel L. and Datura Stramonium L.

Members of the genus other than Datura Stramonium L. have been reported upon favorably and may be found through further investigation to be equally valuable. Datura Metel L. has been shown by Schmidt[3-4] to contain as much hyoscyamin as Datura Stramonium L., also that Datura alba Nees which is grown in China, India and southern Europe for decorative purposes contains hyoscyamin and some atropin in the seed. Hess[5] also found considerable amounts of alkaloid in this species. Peinemann[6] is quoted as having found more alkaloid in the leaf, stem and root of this species than in the corresponding parts of Datura Stramonium L. Kircher[7] examined Datura Metel L., Datura quercifolia and Datura arborea L. finding in the leaves and seeds of quercifolia an average of 0.418 per cent. and 0.292 per cent. of alkaloids respectively. He also noted the presence of alkaloids in Datura Metel L. and Datura arborea L.

The desirability of cultivating and improving Datura Stramonium L. is indicated by an examination of commercial conditions. These conditions as enumerated below indicate a wide range of variations in percentage of alkaloids from year to year and from different geographical sources. Feldhaus[8] in the examination of twenty-five commercial samples from various sources notes a variation of from 0.211 per cent. to 0.495 per cent. with an average of 0.328 per cent. In 1901 he found an average of 0.476 per cent., while in 1902 the average was only 0.337 per cent. Farr and Wright[9] state that in 1906 they found a maximum of 0.30 per cent. and a minimum of 0.12 per cent., average 0.22 per cent. They quote Kordes as having found 0.20 per cent. and Umney 0.39 per cent. to 0.44 per cent. Smith, Kline and French[10] in 1908 report on sixteen assays which indicate a range of from 0.25 per cent. to 0.37 per cent., nine of these being below the United States Pharmacopœial standard. In 1911[11] they again report on fifteen assays ranging from 0.22 per cent. to 0.35 per cent., three of these being below standard, seven not exceeding 0.28 per cent. and five running above 0.30 per cent. Vanderkleed[12] in 1907 reported on nineteen assays ranging from 0.15 per cent. to 0.62 per cent. Again in 1908[13] he considers twenty-five samples of which the lowest was 0.13 per cent., highest 0.51 per cent., average 0.34 per cent. Twenty-one of these were above and four below the official standard, Hankey[14] examined lots which ran as low as 0.14 per cent., with an average of only 0.25 per cent. for the best, Puckner[15] gives a variation of from 0.13 per cent. to 0.45 per cent. as found in sixteen samples. Average results from the Lilly laboratories for the past five years indicate an annual decrease in alkaloidal content: 1907, 0.34 per cent.; 1908, 0.40 per cent.; 1909, 0.38 per cent.; 1910, 0.32 per cent.; 1911, 0.25 per cent.

The foregoing data indicates the desirability of a *uniform high yielding plant.*

The objects of the present investigation have already been stated. The species used were Datura Stramonium L. and Datura tatula L., both introduced into this country. The plants of Datura Stramonium L. were grown from seed purchased in the London market. This seed was not absolutely pure, as one Datura tatula L. plant appeared in the experimental plot from the first planting. The Datura tatula L. plants used in the experiment were transplanted from a vacant lot in Indianapolis. The two forms were grown under the same conditions on soil consisting of stiff clay loam. Cultivation was frequent and continued until mature seed could be obtained.

The samples of leaves for assay from the Datura tatula L. plants were collected August 17th, 1910. At this time the plants bore mature but unripe seed pods, open flowers and numerous buds. August 30th the plot of Datura Stramonium had reached a corresponding stage of maturity and samples were collected on this date. In both cases, individual plants of vigorous growth were selected and numbered. The samples as removed from these individuals were given the corresponding number of the plants and retained separately. Later mature seeds were collected from the same plants. The number of leaves removed was in no instance so great as to interfere with normal growth.

The samples were thoroughly cured at room temperature and stored in paper bags until one year later, when they were assayed. The individual plants assayed as follows:

| | | |
|---|---|---|
| No. B-979 | Datura Stramonium L. | 0.47% |
| No. B-980 | Datura Stramonium L. | 0.55% |
| No. B-981 | Datura Stramonium L. | 0.52% |
| No. B-982 | Datura Stramonium L. | 0.46% |
| No. B-983 | Datura tatula L. | 0.63% |
| No. B-984 | Datura tatula L. | 0.65% |
| No. B-985 | Datura tatula L. | 0.47% |

The process of the United States Pharmacopœia for the assay of Stramonium was used, taking liberal amounts of solvent in all cases, and with the exception that $\frac{N}{20}$ hydrochloric acid was substituted for $\frac{N}{10}$ sulphuric acid in titrating.

It will be noted from the foregoing figures that there is a marked variation in the total alkaloidal percentage of individual plants. Attention has been called to the variation in commercial stramonium for different years and for different geographical sources. Little is known, however, upon the behavior of the percentage of alkaloids in individual plants. True[16] has found that individual belladonna plants vary from 0.2 per cent. to 0.7 per cent. in total alkaloids. The extremes as found for D. Stramonium L. are maximum 0.55 per cent., minimum 0.46 per cent., and for D. tatula L., maximum 0.65 per cent., minimum 0.47 per cent.

Datura tatula L., a species very closely related botanically to the official Datura Stramonium L., indicates a much higher alkaloidal percentage than the U. S. Pharmacopœial species. Both forms considered, likewise show a higher percentage than any commercial drug examined during the past five years, the maximum for this period at the Lilly laboratories being 0.40 per cent., minimum, 0.25 per cent. and average 0.33 per cent. The results obtained also demonstrate that continuous cultivation does not interfere with the natural formation of a high percentage of alkaloids.

The investigation of individual plants from this group is to be continued upon the same plan. Seeds were collected from the plants assayed and those from the highest and lowest yielding plants will be planted and individuals again tested. In one case the object is to bring about an increase in the percentage of alkaloids, while in the other it is to decrease this percentage.

[1] Chevalier: Comptes Rendus. 1910, 150, 344.
[2] Meyer: Arch. der Pharm. 1905, 243, 304.
[3] Schmidt: Arch. der Pharm. 1905, 243, 303.
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[5] Hess: Pharm. Jr. 1900, 64, 250.
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[10] Smith, Kline & French: Anal. Rept. 1908, 36.
[11] Smith, Kline & French, Anal. Rept. 1911, 42.
[12] Vanderkleed: Proc. Penn. Pharm. Ass'n. 1907. 90.
[13] Vanderkleed: Proc. Penn. Pharm. Ass'n. 1908, 88.
[14] Hankey: Am. Drug. 1907, 50, 9.
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[16] True: Oil, Paint & Drug Reporter 1911, 80, 29.

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# POTASSIUM PERMANGANATE IN THE QUANTITATIVE ESTIMATION OF SOME ORGANIC COMPOUNDS

C. M. PENCE, B. S.

Read before the Indiana section of the American Chemical Society, May 10, 1912, and reprinted from the Jour. Industrial and Engineering Chem., March 1913.

Potassium permanganate has been generally used in the volumetric estimation of iron. Some uncertainties formerly existed since it was impossible to obtain a chemically pure article and insufficient data were at hand as to proper methods of preparation and standardization of its solution.

At present, these objections have been largely overcome and almost all of our text books on quantitative analysis contain an extended treatise on proper means of preparation and standardization of volumetric permanganate solutions.

One of the most commonly known organic compounds that is quantitatively determined by the use of volumetric permanganate is oxalic acid. Now oxalic acid and iron are determined in acid solution, but the procedure most applicable for the oxidation of all types of aromatic compounds as well as carbohydrates and hydrocarbons is with alkaline permanganate. Oxidations in acid solution are less energetic than those with alkaline $KMnO_4$ and in the latter case the final product of a completed decomposition of the organic compound is oxalic acid instead of $CO_2$ and $H_2O$.

Among the substances mentioned in the literature (1), (2) and (3) as being oxidized to oxalic acid are propylene, isobutylene, amylene, acetone, fatty acids; butyric, lactic, succinic and tartaric acids; dextrose, sucrose, glycerol and phenol. Now when an organic compound is oxidized to oxalic acid, a further oxidation to $CO_2$ and $H_2O$ readily follows upon acidifying and warming. Such a procedure forms the nucleas of a method for the determination of the compounds previously enumerated. Tocher(3) made use of this method and found that phenol could be determined. His method was substantially as follows: Dissolve 1g. phenol in 1000 c.c. distilled water and take 10 c.c. for titration. Add 3-4 g. $NaHCO_3$ together with a little distilled water. Then add 50 c.c. $KMnO_4$ and boil the liquid for five minutes. Set aside to cool and gradually add dilute $H_2SO_4$ to decided excess; warm to 60 c.c. and titrate the excess N/10 $KMnO_4$ with N/10 oxalic acid.

This method was found to be open to the following objection: that the manganese dioxide formed as a result of the action of N/10 $KMnO_4$ upon the phenol did not reduce readily enough with consequent solution upon direct titration with N/10 oxalic acid. Thus the solution was full of oxide which not only obscured but rendered the end point of little value, in that the oxide was not completely reduced before the permanganate end point was obtained.

The following modification of Tocher's Method was found to give good results:

Dissolve 0.4 g. phenol in 1000 c.c. distilled water. Place 50 c.c. N/10 $KMnO_4$ and 3 to 4 g. $NaHCO_3$ in a 500 c.c. glass stoppered Erlenmeyer flask. Add 25 c.c. of the phenol sol. with gentle rotation. Boil for 5-10 minutes (with stopper removed). Cool flask until temperature is about 60° C. Acidify with dilute $H_2SO_4$, let set about two minutes: cool to room temperature. Dilute with distilled water, add 5 c.c, 20 per cent. KI and titrate the liberated iodine with N/10 thiosulphate solution using starch as indicator. The number of c.c. of N/10 thiosulphate subtracted from number of c.c. $KMnO_4$ originally added = number c.c. of $KMnO_4$ consumed by the phenol.

1 c.c. N-10 $KMnO_4$ = 0.000336 g. phenol.

If a glass-stoppered Erlenmeyer flask is not available an ordinary Erlenmeyer may be used and its contents transferred to a glass stoppered bottle before acidifying. Any oxide adhering to the Erlenmeyer is easily removed by the addition of a little distilled water acidified with $H_2SO_4$ and containing a few drops of 20 per cent KI.

In considering the nature of the oxidation with $KMnO_4$ in acid and alkaline solutions it is observed that each molecule of $KMnO_4$ in acid solution liberates 2.5 atoms of oxygen according to the following equation:

$2 KMnO_4 = K_2O + 2 MnO + 5 O$, or $2 KMnO_4 + 3 H_2SO_4 = K_2SO_4 + 2 MnSO_4 + 3 H_2O + 5 O$.

Now in alkaline solutions the two molecules of MnO are immediately oxidized to 2 $MnO_2$ at the expense of two atoms of oxygen, so that we actually have

$2 KMnO_4 = K_2O + 2 MnO_2 + 3 O$.

Hence for each molecule of $KMnO_4$ used, only 1 1/2 atoms of oxygen are available for our oxidation process. This fact must be recognized in providing sufficient $KMnO_4$ to readily complete the oxidation process and it would necessarily enter into a calculation of the value of N/10 $KMnO_4$ in terms of phenol if the $MnO_2$, or rather its hydrated form, were filtered from the solution before acidifying and adding the KI. But since the procedure was not lengthened by a filtration the $MnO_2$ is reduced to its manganous form with the liberation of free iodine, and we must calculate our factor by considering that reaction proceeds as in acid solution with 2.5 atoms of oxygen avialable per molecule of $KMnO_4$, although such is not literally the truth.

To completely oxidize phenol 14 atoms of oxygen are required, according to following equation: $C_6H_5OH + 14 O = 6 CO_2 + 3 H_2O$.

Since only 2.5 atoms of oxygen are available per molecule of $KMnO_4$ then 5.6 molecules of $KNnO_4$ would be required for every molecule of phenol and the factor for N 10 $KMnO_4$ in terms of phenol becomes

$$\frac{\text{Mol. wt. phenol}}{2.5x2x10x5.6x1000}$$

Experiments with a phenol solution containing 0.0005 g. of phenol, per c.c. as determined by the Koppeschaar bromine method, resulted as follows:

| Exp. No. | Grams Phenol taken. | Per cent. Phenol found. |
|---|---|---|
| 1 | 0.0050 | 99.65 |
| 2 | 0.0050 | 100.18 |
| 3 | 0.0075 | 99.65 |
| 4 | 0.0076 | 99.86 |
| 5 | 0.0100 | 99.86 |
| 6 | 0.0100 | 99.72 |

Now when the cresols were run in the same manner as phenol it was found that they were not completely oxidized and that they varied slightly as to rate with which oxidation proceeded; hence any permanganate method for their accurate determination must depend upon definitely fixed conditions,

Likewise, it was obvious that commercial creosote and guaiacol could not be determined by this procedure, since they are mixtures of several more or less related phenols that are not present in like proportion in different specimens. However, with single solutions of several common phenols and closely related compounds, fairly gratifying results were obtained. Pyrogallol, pyrocatechin, resorcinol and hydroquinone from all of which the $CH_3$ group is absent were readily and completely oxidized.

Benzoic acid was very slightly attacked while under similar conditions, salicylic acid and salol were completely oxidized. Thus it would seem that the phenolic OH group predisposes towards a complete oxidation and that many uninvestigated phenols and closely related compounds would give analogous results. In making up solutions of the several phenols, sufficient N 2 NaOH was added when necessary, to insure ready solution.

The following table is self explanatory:

| Substance— | Per cent. found— |
|---|---|
| Pyrogallol | 100.4<br>100.1 |
| Pyrocatechin | 100.2<br>99.9 |
| Resorcinol | 100.5<br>100.4 |
| Hydroquinone | 99.49<br>99.56 |
| Salicylic Acid | 99.79<br>100.2 |
| Salol | 99.77<br>99.91 |

The alkaline permanganate method is especially applicable for the quantitative estimation of the above compounds when they occur individually in very small amounts in single solutions or in conjunction with substances not readily oxidized.

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*Chemical Research Department.*

## A RECENT ADULTERANT OF MANACA.

F. A. MILLER. B. S.

Reprinted from the Journal of the American Pharm. Ass'n . May, 1913.

The supply of crude manaca on the drug markets of the United States, at the present time, consists largely of an unidentified adulterant or substitute. This form (Fig. II) has recently been noted in all samples examined in the proportions of from seventy-five to one hundred per cent. It is claimed by importers that this substitute is genuine manaca, and that the lighter color of the root is due to a difference of soils in which the drug grows. No significance is given the structural differences which are equally as manifest as that of color. Following is a comparison of manaca with the root noted:

Manaca root (Fig. I) varies in thickness from five to thirty mm. and in length from one dm. to one meter. Externally it is dark reddish-brown. The bark is thin, usually scaly or flaky, and adheres tightly to the wood. It is distinctly bitter. The wood is tough, hard, and of a reddish-yellow color. It is only slightly bitter. The wood is porous, the pores being scarcely visible under a hand lens. The medullary rays are few in number and only visible under a hand lens.

The substitute (Fig. II) varies in thickness from seven to twenty-five mm. and in length from one to four dm. Externally it is yellowish gray. The bark is twice as thick as that of manaca, not scaly or flaky, and seperates readily from the wood. It is practically tasteless. The wood is fragile, slightly softer than that of manaca, and of a pale yellow color. The wood is tasteless. It is porous, the pores being distinctly visible under a hand lens. The medullary rays are numerous, and plainly visible.

*Botanical Department.*

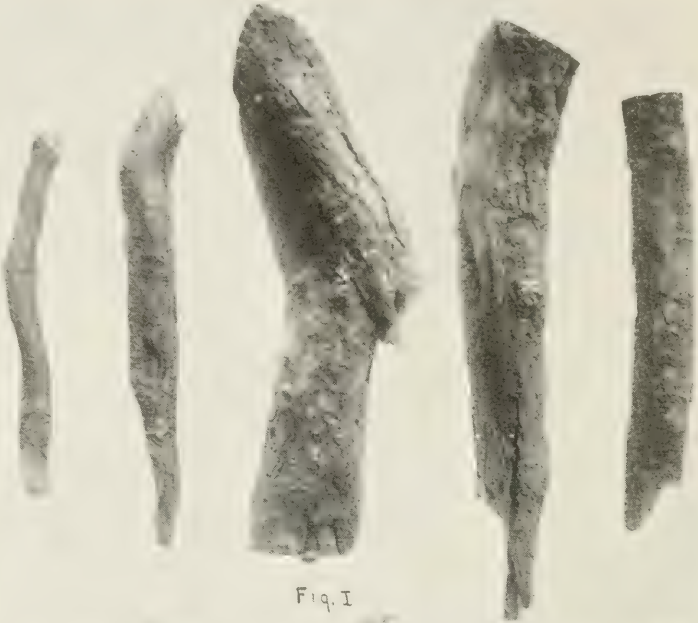

Fig. I

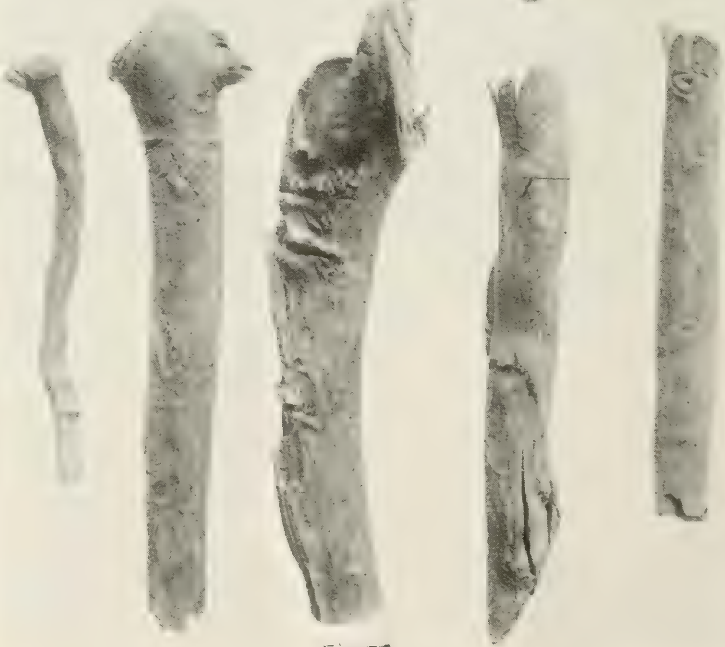

Fig II

Fig. I—Manaca Root. Fig. II—Substitute.

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# THE IMPROVEMENT OF MEDICINAL PLANTS.

## F. A. MILLER

[Read before the Botanical Section, Indiana Academy of Science, Nov., 1912, and reprinted from the Jour. Am. Pharm. Ass'n, June, 1913.]

Certain suggestions were made before the last meeting of the Academy for the possible improvement of valuable medicinal forms through the application of breeding methods. Some of these suggestions have been carried out during the past summer upon experimental plots of belladonna, henbane, stramonium, digitalis and cannabis. The results, though only tentative, are extremely encouraging, and indicate a means of obtaining not only greater yields of the resulting drugs but better and more reliable medicinal products.

Belladonna has shown great uniformity in morphological characters, but considerable variability in the percentage of alkaloids in selected plants. In a comparatively small number this variation was found to be over 50 percent. or from 0.52 percent to 0.87 percent total alkaloids as found in the highest and lowest yielding individuals. Much has been said concerning the variation in total alkaloids as influenced by various conditions. In fact, some experimental work has been done upon the influence of such factors as food elements, light and shade, soils, meteorology, etc., upon the production of alkaloids and other active principles. It now seems apparent, however, that before such data can have any scientific bearing, or be utilized as a means of following the influence of given factors, uniform strains of the plants under investigation must first be obtained. This apparent necessity is due to the wide variations which have been found to exist between the individuals of a given group which have been grown under uniform conditions.

A group of individual plants varying over 50 percent when grown under uniform ecological conditions cannot be expected to behave uniformly when grown under varied conditions. Differences no greater than 50 percent have been reported as being due to certain external influences as affecting all plants upon a given area, while according to recent individual plant investigations, such an area might produce plants varying this much or more among themselves, and representing at the same time any possible mixture with reference to yield. It seems necessary, for this reason, to first obtain a strain of the form under investigation, the individuals of which will react uniformly to certain external conditions. To investigate this point, plants of known alkaloidal yield are being propagated both from inbred seeds and from vegetative cuttings. The progeny thus produced is being grown under the same conditions as the parent plants, as well as under widely different conditions. The alkaloidal yield of these plants will later be taken as a means of determining the results of the various treatments.

The highest yielding individuals from all groups examined are immediately selected as parent plants for possible high yielding strains. The propagation of these favorable individuals is continued throughout the year by means of green-house and cold-frame accommodations, and are tested as rapidly as sufficient material becomes available.

The production of henbane, even upon a small experimental scale, has proven extremely difficult. This difficulty is largely due to the ravages of insects, although cultural difficulties with this plant are not uncommon. It will not reproduce itself from open field sowings and transplants with uncertainty. However, a small number of biennial plants were grown and found to test 0.089 percent total alkaloids at the end of the first season's growth, while commercial drug has only averaged 0.067 percent for the past year. The Pharmacopoeia requires that this drug be collected from plants of the second year's growth. The above figures indicate that it may be entirely unnecessary to grow this plant through the second year to obtain a high yield of alkaloids. The annual form was again observed and though no tests were made, an abundance of seed was obtained from which plantings will be continued. The appearance of this annual form in many plantings of henbane of supposed biennial origin has led to much dispute. Its investigation is necessary from this point of view as well as the possibility for developing an annual form which would possess many cultural advantages over the biennial.

The selecting of high-yielding stramonium plants upon a basis of their contained alkaloids has been continued through two years. Averages as obtained from the progeny of selected parent plants have shown a marked increase over those from wild plants growing in the same locality. These averages are 0.61 percent, 0.50 percent, 0.60 percent and 0.64 percent from Datura Stramonium L., and 0.49 percent, 0.54 percent, 0.62 percent and 0.68 percent from Datura tatula L., as compared with 0.28 percent from wild plants of Datura

Stramonium L., and 0.42 percent from wild plants of Datura tatula L.

Thirty-two forms of the genus Digitalis are under cultivation. These consist of all the species and varieties so far obtainable. These must first be tested for identity before any extensive breeding operations can be performed. Physiological tests have been made which indicate great differences in the toxicity of some of the more accurately named species and varieties. Though more strictly biennial in habit, it has been found possible to bring a number of varieties into flower the same year from seed, thereby shortening any breeding operations by one year.

It has been interesting and valuable to follow the effects of Indiana soil and climate upon the medicinal value of Cannabis Indica. This is an imported drug consisting of the flowering tops of Cannabis sativa L. It always contains more or less seeds of high percentage germination. Repeated tests have been made upon material obtained from plants grown from seed found in shipments of high-testing drug. Without exception these tests have shown a decrease of from 40 percent to 60 percent in value as compared with the original shipments from which the seeds were obtained. One strain has been continued under cultivation in the same locality and upon the same soil for four consecutive years, and its value as indicated by physiological tests has fluctuated between 40 percent and 65 percent. This fluctuation has been intermittent, and not in the nature of a regular annual increase or decrease. During this time, however, a marked improvement has resulted in the size and character of the inflorescence. By selection, this has become heavier, more compact, larger and less leafy. A dwarf form has also resulted which would greatly simplify the process of collection.

Figures No. I and II show some of the experimental plots, and convey some idea of the scale upon which the work is being done. Large numbers of plants are being used, and these are observed throughout the entire growing season before any selections are made. In this manner the entire life history of the plants, from earliest seeding stage to maturity, is made to serve as a record from which intelligent selections can be made.

***Botanical Department.***

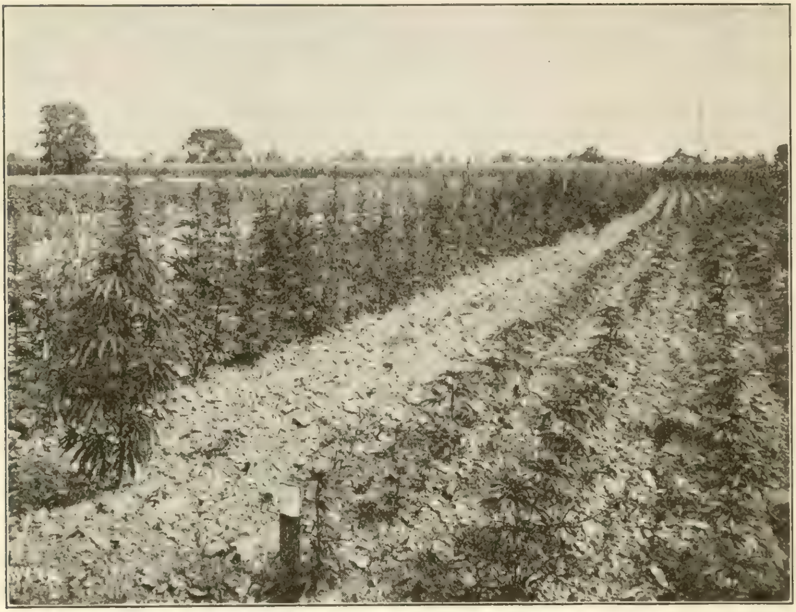

Fig. I. Growing Cannabis from Various Geographical Sources.

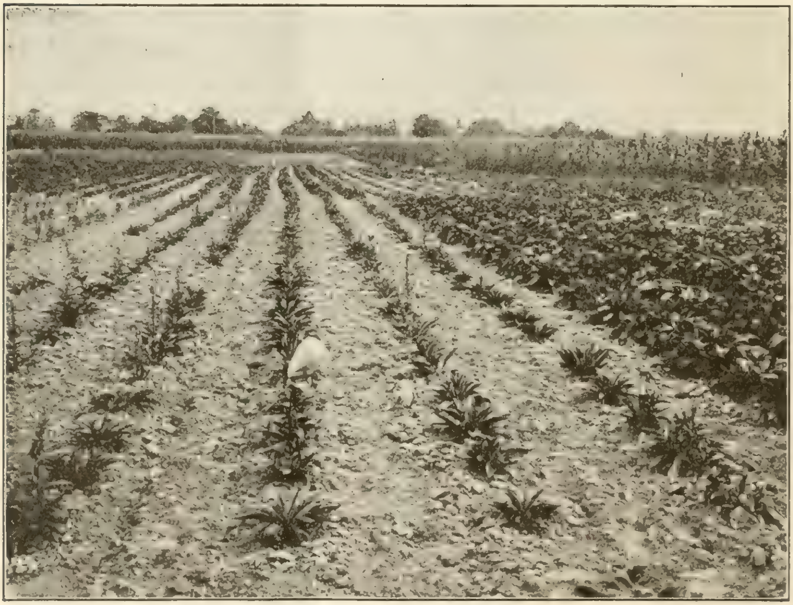

Fig. II, Various species and varieties of Digitalis.

# A CONVENIENT METHOD FOR THE ESTIMATION OF ALBUMIN IN URINE.

FRANK R. ELDRED AND C. M. PENCE

[Read before the Scientific Section of the American Pharmaceutical Association at the meeting held in Denver, Col. August, 1912 and reprinted from the Jour. Am. Pharm. Assoc. February 1913]

The determination of albumin in urine by weighing the precipitated albumin or by determining its amount by the Kjeldahl method, requires so much time that it is not adapted to the needs of the clinician. Several methods have been devised for the approximate determination of albumin, depending upon the measurement of the volume of a precipitate after centrifuging or allowing to stand. Upon trying these methods they were found to give results so unreliable as to be of little value.

As titration methods for albumin did not seem practicable, an endeavor was made to obtain the albumin in the form of a precipitate the volume of which would bear a more constant relation to the amount of albumin present. As it had been observed that acetone precipitated albumin in a flocculent form from aqueous solutions, it seemed probable that this precipitate would settle rapidly and compactly owing to the low specific gravity of the acetone. This was found to be the case with specimens of urine containing albumin, but aqueous solutions of serum albumin yielded a precipitate which did not settle readily, indicating that some other constituent of the urine affected the precipitate. Various constituents of normal urine were tried and it was found that if a small amount of monobasic sodium phosphate was added to the albumin solution, the precipitate would settle as rapidly as from urine. It was soon observed that in order to obtain satisfactory results, the urine must be distinctly acid, and for this purpose acetic acid was added to the acetone.

*Method*—Filter the urine if cloudy and measure 1 cc. from a pipette or burette into a 5 cc. graduated test tube having an internal diameter of 9 mm. Dissolve about 0.04 gm. of monobasic sodium phosphate in the urine and fill the test tube to the 4 cc. mark with a mixture of 98 volumes of acetone and two volumes of glacial acetic acid, both of U. S. P. quality. Close the test tube with a stopper, invert slowly six or seven times and then shake vigorously for thirty seconds. Allow the test tube to stand in a vertical position for exactly fifteen minutes; read off the volume of the precipitate and determine the percentage of albumin by reference to the following table:

| Cubic Centimeters Precipitate | Percent Albumin | Cubic Centimeters Precipitate | Percent Albumin |
|---|---|---|---|
| 0.20 | 0.09 | 0.75 | 0.91 |
| 0.25 | 0.13 | 0.80 | 1.01 |
| 0.30 | 0.17 | 0.85 | 1.10 |
| 0.35 | 0.22 | 0.90 | 1.19 |
| 0.40 | 0.29 | 0.95 | 1.29 |
| 0.45 | 0·37 | 1.00 | 1.38 |
| 0.50 | 0.45 | 1.05 | 1.48 |
| 0.55 | 0.54 | 1.10 | 1.59 |
| 0.60 | 0.64 | 1.15 | 1.72 |
| 0.65 | 0.73 | 1.20 | 1.86 |
| 0.70 | 0.82 | 1.25 | 2.05 |

If more than 1.25 cc. of precipitate is obtained, dilute the urine with an equal volume of water and make a new test, using 1 cc. of the diluted urine, and multiplying the percentage found in the table by two.

In compiling this table, sixteen aqueous solutions of serum albumin varying in strength from 0.1% to 2.0% were prepared. These solutions were standardized in the following manner. The albumin was precipitated with potassium mercuric iodide, heated in a water bath, separated by filtration and determined by the Kjeldahl method using the factor 6.3. About forty determinations were made upon each of these solutions by the acetone precipitation method. A curve was plotted from the average results obtained, and from this the table was constructed. The use of normal urine instead of water in making up the albumin solutions caused no difference in the results; and in a number of pathological specimens gave results which agreed closely with those obtained gravimetrically. While it can not be expected that a method of this kind will give accurate results, yet if carried out with proper attention to details it will be found to give more accurate results than those obtained by other methods based upon the volume of the precipitate.

The results are not influenced by ordinary variations in temperature, nor by the changes in acidity or amount of phosphates caused by the varying composition of different urines.

Considerable variations in the diameter of the measuring tube or in the manner of mixing the liquids were found to affect the results

The precipitate settles so rapidly that after fifteen minutes the volume changes very slowly. For this reason, it did not seem necessary to consider centrifugal separation as a means of shortening the method, although it is probable that good results might be obtained in that way. *Department of Chemical Research.*

# INFLUENCE OF ALCOHOL UPON THE TOXICITY OF DIGITALIS FOR GUINEA PIGS.

CHARLES C. HASKELL, B. A., M. D.

[Reprinted from the Journ. Am. Pharm. Assoc., July, 1913.]

In a recent paper on biological standardization, Eggleston[1] draws attention to the fact that in Hatcher's cat method the presence of alcohol does not influence the results when the digitalis bodies are tested. He suggests that this may not be the case when the guinea pig or frog is used as a test animal. The following experiments were carried out upon guinea pigs and rabbits in the attempt to secure definite information regarding this point. The preparations tested were ordinary U. S. P. tinctures of digitalis, containing originally 48 percent alcohol, in which form they were used in the experiments recorded in Table 1. All injections were made subcutaneously with Hitchens' syringe[2] and the animals were observed for twenty-four hours. It was found convenient to cover the needle hole with collodion to prevent oozing of injected fluid. Doses are given in fractions of a cubic centimeter per gram body weight.

TABLE I.

A. Tests Upon Guinea Pigs.

Tr. No. 1.

| Date | Survived | Died | Minimum Lethal Dose |
|---|---|---|---|
| 6-11-12 ........... | .004 | | .007+ |
| | .004 | | |
| | .004 | | |
| | .0065 | | |
| | .007 | | |

B. Tests Upon Rabbits.

Tr. No. 2.

| | | | |
|---|---|---|---|
| 9-26-12 ........... | .003 | | .008+ |
| | .006 | | |
| 9-27-12 ........... | .007 | | |
| | .008 | | |

Portions of the two tinctures used in the preceding experiments were evaporated to a semi-fluid consistency on the water bath and this residue suspended in a volume of distilled water one-half that of the amount of tincture taken. When the injections were made an equal amount of distilled water was added, bringing the volume dose up to that of untreated tincture.

TABLE II.

A. Tests Upon Guinea Pigs.

Tr. No. 1 evaporated on water bath; residue suspended in distilled water.

| Date | Survived | Died | Minimum Lethal Dose |
|---|---|---|---|
| 6-14-12 .......... | | 0.0080<br>0.0075<br>0.0060 | 0.0045 |
| 6-15-12 ........... | | 0.005<br>0.005<br>0.0045 | |
| 6-17-12 ........... | | 0.0040 | |
| 10- 4-12 ........... | 0.004 | 0.005 | |

B. Tests Upon Rabbits.

Tr. No. 2 evaporated on water bath; residue suspended in distilled water.

| | | | |
|---|---|---|---|
| 9-26-12 ........... | 0.002<br>0.004 | | 0.006 |
| 9-27-12 ........... | 0.005 | 0.006 | |
| 9-28-12 .......... | 0.005 | 0.006<br>0.006 | |
| 9-30-12 ........... | | 0.006<br>0.006<br>0.006 | |
| 10- 1-12 ... .... ... | 0.005 | | |

It is evident from these figures that the original tinctures possessed considerably less toxicity for guinea pigs and rabbits than they did after removal of the alcohol. Several factors must be considered, however, before the assumption can be made that this difference is due to the antagonistic action between digitalis and alcohol. It is possible that heating the tinctures upon a water bath might cause a decomposition of the active glucosides into bodies of greater toxicity. The simplest way to decide this is to make the alcoholic strength of the suspension the same as that of the original tincture, and this was done in some instances by taking up the residue with 50 percent alcohol, in others, by using an equal volume of 95 percent alcohol, when the double-strength aqueous suspension was injected, whereby the suspension was brought up approximately to the original volume and alcoholic strength of the tincture.

TABLE III.

A. Tests Upon Guinea Pigs.

Aqueous suspension from Tr. No. 1 with alcohol up to 48% added.

| Date | Survived | Died | Minimum Lethal Dose |
|---|---|---|---|
| 6-15-12 ............ | 0.007 | | 0.008 |
| 10- 3-12 ............ | | 0.014<br>0.016 | |
| 10- 4-12 ............ | 0.007 | 0.008 | |

B. Tests Upon Rabbits.

Aqueous suspension from Tr. No. 2 with alcohol up to 48% added.

| | | | |
|---|---|---|---|
| 9-28-12 ............ | 0.008 | 0.009 | 0.009 |

From this, it would seem that when alcohol is injected simultaneously with the treated tincture the toxicity of the latter is no greater than that of the original tincture; or, in other words, the heating and drying did not, in themselves, apparently increase the toxicity of the tincture. As further confirmation, one aqueous suspension was compared with the original tincture by means of Cushny's frog-heart method, and the strength found to be practically the same.

Another point, however, is whether this decreased toxicity of digitalis in the presence of alcohol is due to a true antagonism between the two substances or due to some local action of the alcohol. To decide this, a series of injections was made into guinea pigs and rabbits, the aqueous suspension and 95 percent alcohol being used in equal volumes, but the injection sites being different. The results are given in Table IV.

TABLE IV.

A. Tests Upon Guinea Pigs.

Aqueous suspension from Tr. No. 1. 95% alcohol. Separate injection sites.

| Date | Survived | Died | Minimum Lethal Dose |
|---|---|---|---|
| 6-17-12 ............ | | 0.007<br>0.007 | 0.0045 |
| 10- 3-12 ............ | | 0.006<br>0.008 | |
| 10- 4-12 ............ | 0.0035<br>0.0040 | | |

B. Tests Upon Rabbits.

Aqueous suspension from Tr. No. 1. 95% alcohol. Separate injection sites.

| | | | |
|---|---|---|---|
| 9-30-13 ............ | | 0.007<br>0.007<br>0.008<br>0.008<br>0.006<br>0.006<br>0.006 | .006 |

From these results it would seem that the protective power of alcohol against poisoning by digitalis is due to some local action of the alcohol, because when the digitalis solution and the alcohol are injected into different spots the former appears to have fully as much toxicity as where no alcohol is used. From appearances at the end of 24 hours, the digitalis seemed entirely absorbed whether alcohol was present or not.

It is clearly evident, however, that when digitalis preparations are tested by subcutaneous administration to guinea pigs or rabbits, account must be taken of the alcoholic content of such preparations, because the results will be markedly influenced by the presence of considerable amounts of alcohol.

Experiments, which will soon be published, have been carried out upon frogs, using the one-hour method, and it has been found that alcohol to the amount of 25 percent has no influence upon the results. The dilution of most galenical preparations necessary for testing by Cushny's method reduces the alcoholic contents to this figure when normal saline or water is used as a diluent.

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1. Eggleston: Am. Jr. Pharmacy, Vol. 85, No. 3, page 99.
2. Hitchens: Journal of Exper. Med., Vol. VIII, page 646.

*Dept. of Experimental Medicine.*

# BREEDING MEDICINAL PLANTS.

F. A. MILLER, B. S.

[Read before the Plant Section of the American Breeders' Association at the meeting held in Columbia, South Carolina, January 25, 1913, and reprinted from American Journal of Pharmacy, July, 1913.]

The products from medicinal plants are without doubt as valuable to mankind as those from the cereal, vegetable, fruit, flower, fibre and other economic plants under cultivation. The latter have all yielded to the principles of plant breeding, and have supplied man with a wealth and variety of products which nature's laboratory has never equalled. Why should not medicinal plants yield and produce in a similar manner, and through cultivation and improvement be made to furnish mankind with more efficient remedies against disease?

An examination of the crude vegetable drugs as they occur on the drug markets of to-day reveals a mass of inferior materials far in excess of what might be expected. Much of this material is unfit for manufacturing purposes, through adulteration with unknown and worthless admixtures, partial or complete substitution of one plant or plant part for another, old, inert, mouldy drugs which may have been stored under adverse conditions or collected out of season and improperly cured and packed. All this is due to a lack of power to control the production of crude vegetable drugs. Too much must be left to nature or to none too well informed collectors. The faults and inefficiencies of nature need little comment. A comparison of a few improved varieties with their wild ancestors is sufficient evidence that nature is poorly equipped for the production of improved strains.

Ignorant collectors many times are a menace. Personal experience with many of them has revealed an absence of any sense of responsibility, and but little power of discrimination in selecting and identifying plants. They cannot separate closely related species, a procedure often necessary in the intelligent collection of medicinal plants, and frankly refuse to observe certain rules governing collection and curing. It is thus evident that the variations of nature associated with ignorance, often give the pharmacist and practicing physician a poor and suspicious product. Rigid inspections must be enforced at all stages in the process of manufacturing medicinal preparations. But however rigid these inspections may be, they cannot overcome all the variations of plant growth or correct all the mistakes of careless collectors. The supply of medicinal plant products should be controlled with the same degree of nicety as the agricultural products or even with greater precision, since in many instances a life is dependent upon the strength and purity of some vegetable drug.

Plant breeders are supplying fruits of varying acid values, corn of high and low percentage of oil and protein, carefully selected sugar beets of high yielding power, and varieties of tobacco suitable for various purposes according to an indicated nicotine content. All of these achievements and numerous others are noteworthy. Of a different character, but of no less importance are the drug producing plants which yield the alkaloids, glucosides, saponins, resins, oleoresins, etc., upon which their curative property depends. Cannot the plants yielding these so-called active principles be brought under the influence and control of the breeder, and be made to produce their respective products more abundantly and more consistently than in the wild state? In attempting to answer this question, experiments have been started with several medicinal plants which will extend over a considerable period of time, and involve various problems of selection and breeding.

The *Solanaceæ* offer as rich a field in the development of improved medicinal forms as it has already offered in the production of the potato, tomato, egg plant and capsicum among the food producing plants, and the datura, solanum, capsicum and tobacco of the decorative forms. In the terminology of the druggist there is found within the same family the very important form, belladonna, henbane and stramonium, all yielding alkaloids and readily amenable to chemical methods of assay. These three genera, in addition to others from different plant families, are being used in the above mentioned experiments. Chemical and biological methods are being used in checking and following the progress of the work. It is hoped that correlations may be found to exist between high potency and certain morphological characters. This would eliminate in part at least the chemical and physiological assays, which are expensive, and somewhat long. Following is a brief discussion of what is being done with some of the forms under investigation.

## BELLADONNA.

Both the leaves and roots of belladonna (*Atropa belladonna*) are used. They must yield respectively 0.30 per cent. and 0.45 per cent. of alkaloids. During the past three years, 6 per cent. of all shipments of the leaf examined were below standard. The variation in percentage of alkaloids for the same period of time was from 0.23 to 0.62 per cent., average 0.43 per cent. Of the shipments of root examined, 28 per cent. were below standard, with a variation of from 0.17 to 0.66 per cent., average 0.48 per cent. No attempts have been made to breed belladonna for a high yield of alkaloids, a possibility which is suggested by the range of variations as indicated above. That the belladonna plant does possess a higher yielding power than average figures would suggest, is shown by a yield of 0.9 per cent. of alkaloids which was obtained upon a plot fertilized with commercial acid phosphate. Such a high yielding power as this would of course not be transmitted by these plants to their offsprings. It is only mentioned to indicale the possibilities of locating high yielding plants by testing leaves from selected individuals.

During the past summer a number of such selections were made. Individual plants were selected, numbered and inbred. Samples of leaves were taken from these individuals upon which to determine the alkaloidal yield. These selected plants were also propagated vegetatively and the resulting plants are being grown in the greenhouse. Some of them are growing in pots in the original soil in which the parent plants were grown. while others are growing in various mixtures of widely differing soils. In this manner it is hoped that some information may be gained upon the behavior of the alkaloids with respect to inheritance, effects of soils, variation in yield from plants grown from open pollinated and close fertilized seeds, and from those propagated by cuttings. The selected plants so far tested indicate a variation in yield of from 0.55 per cent. to 0.87 per cent. of alkaloids. The progeny of these plants of known yield will be tested in a similar manner.

The external characters of the belladonna plant are extremely uniform, with the exception of total yield of leaves and roots per plant. This exception will be taken advantage of in selecting for increased production of these products. Individuals vary in amount of dry root produced from 139 grams to 203 grams. It has been stated that the percentage of alkaloids in the roots of this plant increase markedly after the first year, and reach a maximum at the end of the third year's growth. Belladonna is not perfectly hardy throughout the central United States, and more hardy strains must be developed before the above condition can be observed to advantage. Sixteen hundred plants are now being tested in the vicinity of Indianapolis for relative hardiness. See Figure I, Page 134 for test plot of belladonna.

## HENBANE.

Henbane is a pharmacopœial drug, supposed to consist of the dried leaves and flowering tops of *Hyoscyamus niger* collected from plants of the second year's growth. This product must yield not less than 0.08 per cent. of alkaloids. Records covering one hundred and two inspections of this drug purchased in the drug markets of the United States, show but thirteen per cent. with a yield of alkaloids equal to or above this requirement. The remaining eighty-seven per cent. vary from 0.018 per cent. to 0.075 per cent. From a botanical point of view, this drug is also far from uniform. Many samples and shipments contain seeds which germinate readily, and when grown to maturity, furnish a means of accurately identifying the original material. A number of shipments have been checked in this manner during the past two years, and annual plants have been found in nearly all cases. The official requirements state definitely that the drug must be collected from plants of the second year's growth. However, without some provision for controlling this collection, little can be done toward obtaining an official product in this respect. Certainly the above conditions of alkaloidal yield and botanical origin of this drug are strongly suggestive of the necessity and desirability of subjecting the genus to a thorough and rigid investigation. This investigation should have to do with the isolation and cultivation of the annual and biennial forms, as well as all species and varieties of these. Individual plants should be selected for breeding purposes, and tested for yielding properties in the same manner as described for belladonna.

## STRAMONIUM.

Stramonium has been taken up in a similar manner, and the work on *Datura stramonium* and *Datura tatula*, two common forms, has now been carried through the second year. Selections of *Datura tatula* gave a variation in alkaloidal percentage of from 0.47 to 0.65. The plants yielding these extremes produced offsprings as follows:

Of ten individuals from the plant yielding 0.47 percent. alkaloids, a range of from 0.44 per cent. to 0.57 per cent. was obtained, the average for the ten being 0.51 per cent. Of the same number of individuals from the plant yielding 0.65 per cent., a range of from 0.43 per cent. to 0.77 per cent. was obtained, the average in this case being 0.65 per cent. In the first group seven of the ten plants tested exceeded the parent in alkaloidal yield, while in the second, only five exceeded the parent. It is of interest to note that the lowest limit (0.43 per cent.) was found in the progeny of the high yielding plant (0.65 per cent.), the lowest limit in that of the low yielding plant (0.47 per cent.) being 0.44 per cent. The most promising feature of this experiment in its present stage is the greater average yield obtained over that from wild plants from the same locality. A mixed sample of leaves from uncultivated plants of *Datura tatula* gave a yield of only 0.35 per cent. in comparison with average yields of 0.51 and 0.65 per cent. from selected plants. These latter figures might also be compared with the average yield of commercial shipments of stramonium as noted for three years, which is 0.34 per cent. The analysis of the *Datura stramonium* selections of the past year, which were performed in the same manner as those of *Datura tatula*, have not been completed. The parent plants, however, from which these selections were made, gave yields of 0.46 per cent. and 0.55 per cent. respectively, which figures represent the low and high limits obtained from a number of individuals.

Two other varieties of stramonium not common to this country were grown and tested. These were *Datura humulis flava*, bearing large, beautiful, double yellow flowers of peculiar fragrance, and *Datura ferox*, a form very closely resembling *Datura tatula*, but having a more vigorous and robust habit. Both of these forms were obtained from Germany. The first contained in a mixed sample, 0.42 per cent. of alkaloids, and individual selections of the second gave a variation of from 0.53 per cent. to 0.70 per cent. of alkaloids.

It is to be regretted that none of the first plants selected for testing were close fertilized. During the past year, all selected plants were inbred, and only these will be used in continuing the work. Twenty crosses were made among the three species, *stramonium, tatula* and *ferox*. The effects of these crosses upon alkaloidal yield as well as upon visible characters, will be noted during the next growing season. See Figure II for breeding plot of stramonium.

## DIGITALIS (Foxglove).

Digitalis, the common garden foxglove, has been chosen as another medicinal plant upon which to test the effects of breeding. It is also an official drug, and must consist of the leaves from the second year plant of *Digitalis purpurea* at the commencement of flowering. This form has been included for experimental purposes on account of its value to the physician and because of a wide variation and much uncertainty in physiological effect. There is also a lack of experimental data upon such questions as the comparative value of the wild and cultivated plant and of the many different species and varieties, of the effects of cultivation upon medicinal value, time of collection, methods of curing, packing and storing and of the influence of various ecological factors.

In the study of the group, it is not only desirous to compare the many species and varieties medicinally, but also to determine their relative yield of crude material, ease of culture, hardiness, flowering period and effects of hybridization upon these respective characters.

Thirty-two forms consisting of both species and varieties, are under observation. These have been started from seed purchased of commercial seedsmen. The most prominent trade catalogues from this country, England, Germany and Japan have been examined, and all forms of the foxglove listed in them have been obtained. Some of these were started in the green-house as early as December in an effort to bring as many of them as possible into flower the first year. The following table shows the date planted, number of plants in flower on given dates, and total number of plants, both flowering and non-flowering, at end of growing season:

DATES OF FLOWERING AND NUMBER OF PLANTS IN FLOWER.

| Variety | Source | Date Planted | July 15 | July 20 | July 25 | July 30 | Aug. 10 | Aug. 20 | Sept. 1 | Sept. 20 | Oct. 15 | No. Plants Used |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Digitalis lanata..... | Horsford, Vt. | 12-20-11 | .. | .. | .. | .. | .. | 1 | .. | 2 | 2 | 97 |
| Digitalis ambigua.. | Horsford, Vt. | 12-21-11 | 1 | 17 | 35 | 49 | 54 | 87 | 151 | 217 | 220 | 233 |
| Digitalis canariensis | Watkins & Simpson, London | 1- 4-12 | .. | .. | .. | .. | .. | .. | .. | .. | 2 | 224 |
| Digitalis gloxiniæflora lutea...... | Boddington, N. Y. | 1-30-12 | .. | 1 | 1 | .. | .. | 3 | 11 | 68 | 95 | 386 |
| Digitalis purpurea rosea ........... | Boddington, N. Y. | 2- 6-12 | .. | .. | .. | .. | .. | 1 | .. | .. | .. | 358 |
| Digitalis purpurea alba............. | Boddington, N. Y. | 2- 6-12 | .. | .. | .. | .. | 1 | .. | .. | .. | .. | 334 |
| Digitalis gloxinioides ............ | Horsford, Vt. | 2- 7-12 | .. | .. | .. | .. | 4 | .. | 5 | 9 | 10 | 383 |
| Digitalis monstrosa | Watkins & Simpson, London | 2- 7-12 | .. | .. | .. | .. | 1 | 2 | .. | .. | 2 | 319 |
| Digitalis grandiflora | Dreer, Philadelphia | 2- 7-12 | .. | 1 | 2 | .. | 5 | 32 | 113 | 189 | 239 | 240 |
| Digitalis sibirica.... | Horsford, Vt. | 2- 8-12 | .. | .. | .. | .. | .. | .. | .. | 6 | 20 | 294 |
| Digitalis Ivery's spotted......... | Ferry, Mich. | 2-29-12 | .. | .. | .. | .. | .. | .. | .. | .. | 1 | 244 |
| Digitalis sp?........ | Yokohama, Japan | 3- 5-12 | .. | .. | .. | .. | .. | .. | .. | .. | 5 | 239 |
| Digitalis macranthis | Benary, Germany | 3-22-12 | .. | 2 | 3 | .. | 16 | 81 | 161 | 217 | 222 | 231 |
| Digitalis lutea...... | Benary, Germany | 3-22-12 | .. | .. | .. | .. | .. | .. | .. | 1 | 3 | 256 |
| Digitalis buxbaumi. | Benary, Germany | 2-22-12 | .. | .. | .. | .. | .. | .. | 1 | 7 | 25 | 319 |

The early flowering individuals noted in the table are being utilized for breeding purposes, in the hope of obtaining either annuals or biennials of a higher and more uniform quality. Mixed samples of leaves collected from plants of the first year's growth of all varieties studied have been biologically tested. Many of the varieties test equally as high as good commercial drug, and some of them even exceed this article in relative strength, as indicated by the above method. Others have proven extremely inactive, the poorest, as indicated by the physiological tests, being only one-sixth as active as the best.

In addition to the biological tests, the external characters must also be closely observed. Upon a basis of leaf forms, the genus is easily divided into two groups. One of these is characterized by broad, rough leaves and includes such varieties as *purpurea, monstrosa, alba, gloxinioides* and others. They vary greatly in physical characters, and apparently hybridize with considerable ease. The other group is characterized by narrow, smooth leaves and includes such forms as *lanata, ambigua, grandiflora, sibirica, canariensis* and others. The members of this group vary little in external characters, and hybridize with considerable difficulty.

The diversity of leaf forms, as noted for individuals of the same species or variety, is indicated for two forms, in Figure III. Each leaf was taken from a different plant. The variations in size, shape, margin, petiole, surface and color seem too great and diversified to be explained as individual variabilities. Breeding and the examination of a large number of plants will evidently clear up this point.

The foregoing is only expected to serve as a suggestion to those who may be interested or have the opportunity to observe or investigate medicinal plants. Much good will have resulted if better crude drugs of vegetable origin can be produced from the wild forms, by an application of the rapidly advancing views of the practical breeder. It is only just that the demands upon the plant kingdom should be exhausting, and such will not be the case until medicinal plants are included in the category of the plant breeder.

*Botanical Department.*

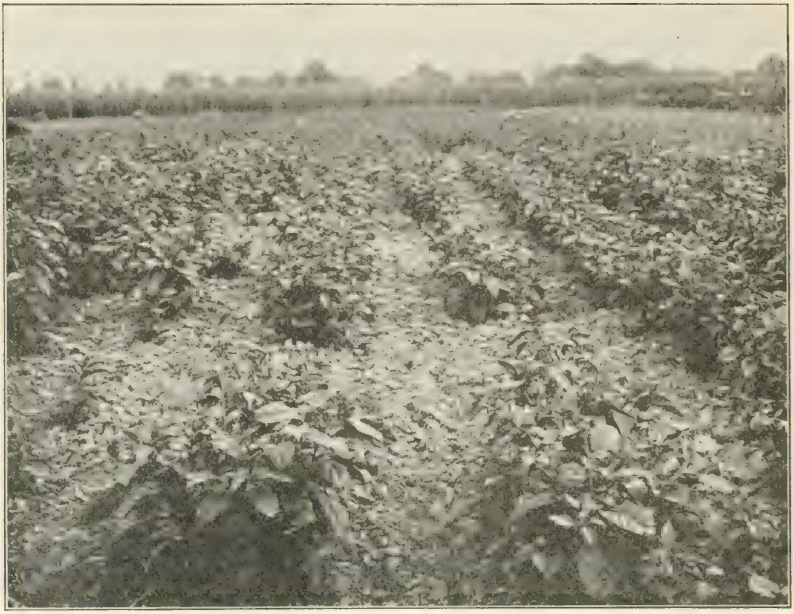


FIG. I. Commercial test plot of Belladonna.

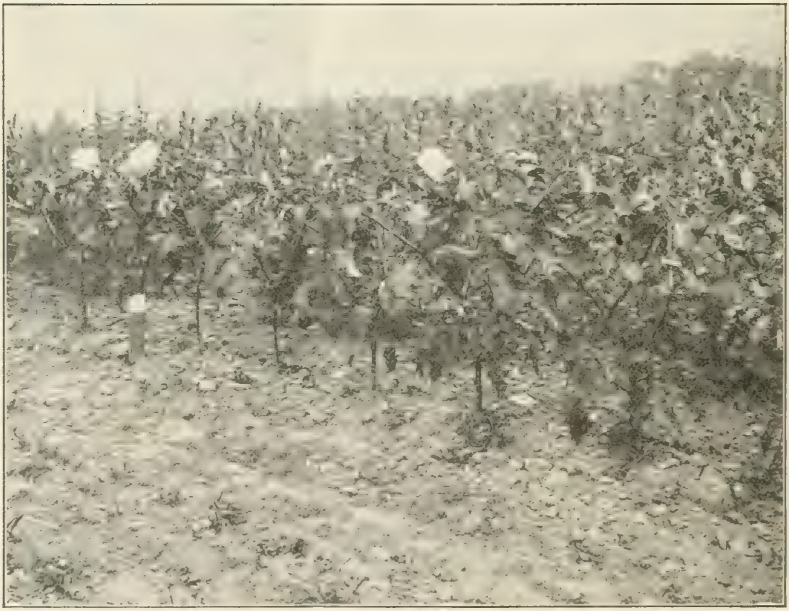
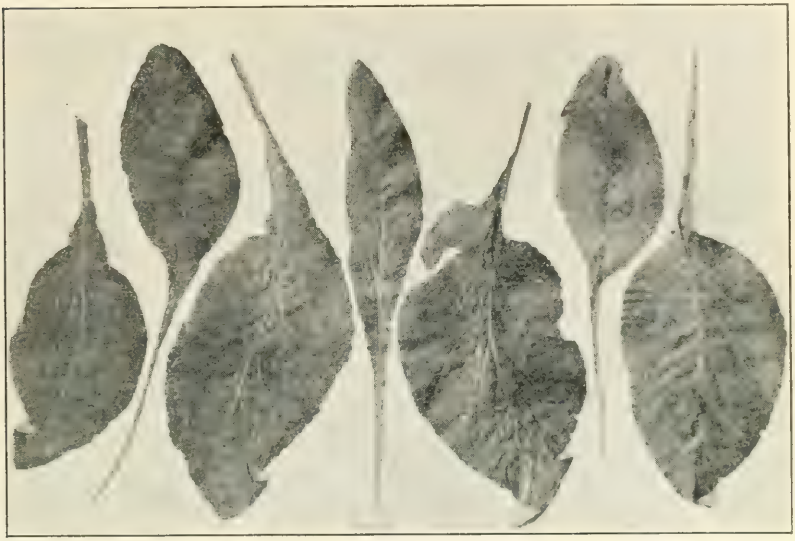
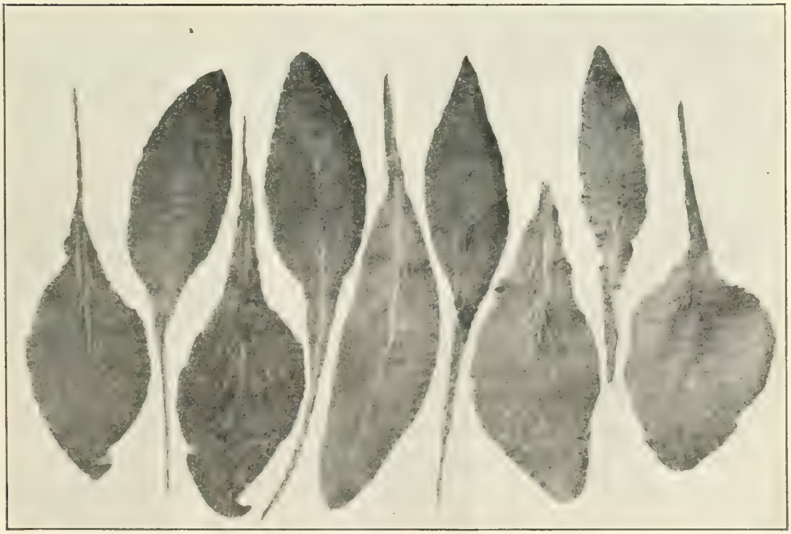


FIG. II. Breeding plot of Stramonium

FIG. III. LEAF VARIATIONS IN DIFFERENT PLANTS OF THE SAME SPECIES OR VARIETY. A TYPICAL MATURE LEAF WAS COLLECTED FROM EACH PLANT.



*Digitalis purpurea.* Watkins & Simpson, London.



*Digitalis gloxinioides.* Horsford's Nurseries, Vt.

# THE COMPARATIVE VALUE OF VARIOUS SUGARS IN THE FEEDING OF INFANTS.

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While it has been claimed that the addition of carbohydrate to the milk modification used in the artificial feeding of young infants is unnecessary, most pediatrists still believe that such addition is desirable. The dilution necessary to reduce the fat of whole cow's milk to a percentage safe for feeding to very young babies causes such a diminution in the amount of sugar that the food administered must either be lacking in the necessary caloric value, or else be fed in such amounts as to overtax digestion and assimilation, certainly of fat, and probably of protein. The question of the routine employment of relatively large amounts of protein is still an open one, but even those who contend that casein of cow's milk never causes digestive disturbances recognize that carbohydrates, in moderate amounts, not only are easily assimilated and burned, but have an important relation to water retention, to fat utilization, and as nitrogen sparers.[19]

If carbohydrates are of value, what form should we select? These substances may, for our purpose, be grouped into polysaccharides, of which starch is a representative member; the disaccharides, such as lactose, saccharose and maltose; and the mono-saccharides, dextrose, fructose, galactose, etc. In the normal processes of metabolism it seems that all carbohydrates before utilization must be converted into dextrose.

If poly- and disaccharides must be converted into dextrose, why not spare the digestive power of the infant by furnishing its sugar in the form of dextrose? While dextrose is easily assimilable, the fact that bacteria rapidly ferment it, with the result that a portion is thereby lost, and, what is of even more importance, the formation of irritant fatty acids in excess, renders it unsuitable for routine use in the infant's food.[8] Moreover, it is conceivable that this ready assimilability may be rather a drawback, in that the intermediary metabolism runs the risk of being overwhelmed by the sudden excess of sugar.

If we turn to the other extreme and consider the polysaccharides, we find that these bodies are not liable to bacterial fermentation until they are split up into the simple sugars.[8] This process is such a gradual one that absorption of the sugars usually prevents fermentation to any great extent. Moreover, it has been shown that older views concerning the inability of the newborn infant to digest starch were erroneous, for a diastasic enzyme is present even in intrauterine life.[11] As a consequence, some authorities recommend the use of cooked starch early in the infant's existence. It seems to be the opinion of most, however, that any considerable amount of starch is almost certain to cause disturbances, so even those who recommend its use, employ, in addition, some disaccharide in order to bring the carbohydrate content of the food up sufficiently high.

As long ago as 1877, Jacobi protested against the addition of lactose to the infant's food. He believed this sugar to be especially liable to split up into the irritant fatty acids, and urged the use of cane sugar and starch decoctions. Cane sugar has rather fallen into disuse of late, however, some considering it inferior to lactose, and nearly all, apparently, believing maltose preferable.

Pure malt sugar or maltose is too expensive to permit of its routine use in feeding. The commercial preparations consist of mixtures of the disaccharide, maltose, with varying amounts of the dextrins, which are polysaccharides, and it seems rational to adopt Leopold's[4] and Southworth's[6] nomenclature, and designate them collectively as the "maltose-dextrin" preparations. It is evident that, strictly speaking, they are not sugars, but for the sake of simplicity they will be spoken of as such. Commercial lactose, on the other hand, consists of one sugar without appreciable admixture of any other substance.

That pediatrists are by no means agreed upon the relative merits of these sugars is shown by referring to some of the standard textbooks. Rotch,[21] Holt[12] and Czerny[24] advocate the use of lactose, while Finkelstein and his followers[23] hold that the maltose-dextrin mixtures are preferable. It is of interest briefly to review certain theoretical considerations which have been advanced in favor of the selection of one or the other of these sugars.

Lactose is always present in the breast milk, and is the organic constituent which is nearly constant in its percentage. It is found in the milk of all mammals and can be prepared in a state of purity as regards chemical composition and bacterial contamination. It is slowly fermentable, being broken up in the intestine (or intestinal walls) and so gradually absorbed as to prevent an overwhelming of the intermediary metabolism with the products of its digestion. Few bacteria but those normally present in the intestines of the normal baby can utilize milk sugar, while "many can utilize maltose."[3]

Herter and Kendall[15] have demonstrated the feasibility of altering the character of the intestinal flora by changes in the diet. Moreover, Theobald Smith[10] has shown that the bacilli of tetanus and diphtheria produce more or less toxin, according to the nature of the media upon which they are grown; and Kendall[10] observed a complete change in the biologic activities of the colon bacillus when dextrose was added to the culture medium. Lactose seems to favor the development of bacillus bifidus, the chief organism found in the colon of the normal breast-fed infant, while maltose encourages the growth of bacillus acidophilus, which, "if present in large numbers, is liable to produce an excessive degree of acidity, which may cause irritation of the intestine and an intolerance for sugar."[3]

If such be the case, should we not endeavor to encourage the formation and maintenance of the "normal" intestinal flora, rather than administer such articles of food as are apt to favor the growth of organisms whose activity may give rise to serious disturbances on the part of the host?

In feeding an infant on modified cow's milk, a food is employed which differs in many important particulars from breast milk. Whether we use simple dilutions of whole milk or top-milk mixtures and split proteins, the fact remains that qualitatively the fat and casein are not identical with those present in mother's milk, nor is the proportion of salts the same. Yet thousands of artificially-fed infants have been successfully reared, not only under the observation of competent pediatrists, but by the use of foods of apparently very unsuitable composition. Such an event is due to the ability possessed in a certain degree by the digestive system to adapt itself to artificial conditions; a faculty which has been demonstrated by animal experimentation.[16] Therefore, it may be urged that the mere presence of lactose in breast milk is no sound argument for its use in modified cow's milk. We do not attempt the impossible task of reproducing the infant's natural food, but rather to furnish such nourishment as will enable it to thrive, and it seems quite possible that under the necessarily artificial conditions some other sugar may be more desirable than that present in breast milk.

What is the significance of the bacterial flora present within the intestine? Are we to assume that a certain group of organisms is characteristic for each species of animal, or for each age of man, and that unless a diet suited to the demands of these bacteria is administered, a group of "foreign" bacteria appears, whose activities are fraught with dire consequences to the host? Or, rather, is the nature of the flora conditional only upon the food taken by the host, and the disturbance which occurs due to the fact that excessive amounts of particular food elements are furnished, and the unutilized portion remaining in the intestine is split up into injurious substances?

If the former be the case, it is hard to understand why favorable results are so often secured on foods which must, if Kendall's views be accepted, change the nature of the intestinal flora. Infants have thriven on simple dilutions of whole milk with low sugar; others on top milk mixtures and high sugar. Even more convincing evidence is offered in the reports on the use of "eiweissmilch,"[13] where the protein is often higher than the sugar, and in Holt's[2] "synthetic milk" the protein content is often as high as 5 per cent. Nor have Kendall's claims been verified by other investigators. Otaki and Vogt,[18] experimenting on puppies; Friedlander and Greenbaum[7] on marantic infants; Veeder, Kilduffe and Denny[1] on patients with acute gastrointestinal disease; and Wollstein[2] on babies with chronic intestinal indigestion, saw no such marked and striking change as that observed by Kendall. Veeder[1] suggests that the suppression of pernicious bacterial activity was the result and not the cause of his patients' improvement.

Do the maltose-dextrin preparations possess any merits which render them preferable to lactose in feeding infants? If we accept the statements of many that advocate the use of maltose-dextrin, lactose should never be employed, because its assimilation limit is less than one-half that of maltose; because lactose is more apt to cause diarrhea; because maltose-dextrin causes a more rapid gain in weight; and because maltose is more readily assimilable.

A little reflection makes it evident that some enthusiasts are attributing to the commercial preparations all the virtues of pure maltose and, in addition, those due, probably, to the presence of the dextrins. This will be brought out more fully in the consideration of the especial points.

First, in regard to the "assimilation limit." We are told that the assimilation limit for lactose, as determined by Grosz,[14] is from 3.1 to 3.6 grams per kilogram body weight, while the assimilation limit for maltose, according to v. Reuss,[11] is over 7.7 grams. What does this "assimilation limit" mean? A perusal of Grosz's paper shows that his conclusions on this point were drawn from experiments upon 5 healthy and 3 sick infants, eleven to thirty-four days old; and upon 6 children from one to six years old. His procedure was to administer a 25 per cent. solution of lactose in one dose, or, when quite large amounts were given, in two doses within an hour, to breast-fed infants or to older children on simple solid diet. Obviously, the infants were receiving considerable amounts of lactose in the ingested milk. Having learned the amount of milk taken in a series of 13 cases, and assuming a sugar content of 5.91 per cent. for breast milk, Grosz estimates that the infants observed took from 4.7 to 12.5 grams of lactose per kilogram in twenty-four hours without the appearance of lactose in the urine. Consequently, it was necessary, in some cases, to give as much as 16.2 grams of lactose for every kilogram of body weight before the assimilation limit was over-stepped. But it was found that less than 3.1 grams of milk sugar in 25 per cent. solution at one dose failed to cause lactosuria. Therefore, could it not be assumed that the administration of, say, 3 grams every hour in addition to the sugar present in breast milk would not cause the elimination of lactose in the urine? The clinician is interested in knowing how much lactose he can give safely in twenty-four hours, not how much can be tolerated in concentrated solution by an infant already receiving fully as much as would be given in artificial feeding.

Moreover, what are the consequences of over-stepping the boundary set by the mysterious "assimilation limit"? Lactose appears in the urine, therefore lactose must have been circulating in the blood. But that lactose in the blood is a menace to health and life remains to be proved. Lactosuria frequently occurs in pregnant women, and is, according to Williams,[20] "without significance." Grosz[14] says: "In conclusion I might mention only that after milk-sugar feeding to infants we could perceive absolutely no disturbance on the part of the digestion. The infants gained regularly and the stools passed showed no change in their consistence. . . Here and there the number of stools were somewhat increased; in their consistence, however, they were fully normal." Finally, Nothmann[17] has reported that lactose frequently occurs in the urine of premature infants, in spite of which fact the infants show no signs of discomfort, but develop normally.

Apparently, the "assimilation limit" for lactose as determined by Grosz and Nobecourt[22] has no practical importance as regards the rational feeding of milk sugar to normal infants. Considerably more may be split and assimilated than is ever given, and even should the limit be over-stepped, dire consequences do not seem to ensue.

In the normal breast-fed baby, a certain amount of lactose is split up into fatty acids and other substances. This means that a portion is lost so far as the carbohydrate metabolism is concerned, and also that bodies of an irritant nature to the intestine are produced. Normally the irritation is so slight as to do no more than act as a stimulant to peristalsis, but when excessive amounts of lactose are fed, or where a lactose intolerance exists, the irritation becomes so pronounced that enteritis and diarrhea are the consequences. It must be remembered, however, that maltose may undergo a similar fermentation, so on theoretical grounds there seems little in favor of the latter so far as regards the production of diarrhea. But, it is urged, there are present, in addition to maltose, the non-fermentable dextrins in commercial maltose-dextrin preparations. The long-established practice of adding barley water to milk modifications containing lactose furnishes non-fermentable polysaccharides, which should be fully as efficient as the dextrin accompanying maltose.

Leopold[25] observed the action of various sugars on young infants and found that lactose was more prone than saccharose to cause diarrhea, while maltose-dextrin rarely produced this disturbance. It is probable that the amounts of the different sugars were the same and that they were administered in the similar concentration in his experiments. His only statement in this respect is: "The sugar was in an amount of 15-30 g. dissolved in 100 c.cm. of distilled water, and given at one time between two feedings in the evening." Granting that, under the conditions present in the experiment, lactose was more prone to cause diarrhea, does this have much bearing on the use of sugars in feeding? Leopold tells us: "The children's food consisted either of buttermilk *with sugar addition* or in half milk with about 4 per cent. Mehemalzsuppenextrakt or breast and buttermilk was given." Therefore, the infants received nearly 5 per cent. lactose "with sugar addition"; or 2.25 per cent. lactose and 4 per cent. malt extract; or 5 to 6 per cent. lactose. Now, added to this, there is given a considerable amount of lactose in concentrated solution. Small wonder that, under these circumstances, virtues become vices and, what under normal conditions, would be advantageous in lactose becomes, under these palpably artificial conditions, distinctly the reverse.

As regards the gain in weight, that observed shortly after the administration of large amounts of sugar probably depends upon the lessened excretion of water, and is of little importance as to growth and development, though it may be of significance in those instances where previous water loss has been excessive (intoxication). All experiments tend to show that maltose-dextrin acts more efficiently in this respect than does lactose.

The more gradual and steady gain which we generally associate with satisfactory progress, when accompanied by normal stools and signs of content, seems also more satisfactorily secured by use of the malt sugars, if we base our conclusions on results secured in "difficult feeding cases." Ladd[5] has had occasion to observe this superiority of the maltose-dextrin preparations; and Leopold[4] presents even more striking evidence.

But we should not be too hasty in assuming that, because Ladd's and Leopold's patients gained better on maltose-dextrin than they did on other sugars, lactose and saccharose are no more to be employed. Until quite recently maltose-dextrin preparations were practically unknown, even to most pediatrists in this country. It is reasonable to assume, then, that these children had previously received lactose or saccharose, and had either failed to gain, due to some individual peculiarity, or had acquired a sugar intolerance from receiving excessive amounts. What is more natural than that they should do better on some other sugar?

Maltose may pass directly into the blood stream, and there be split up into its constituent dextrose molecules; maltase, the maltose-splitting enzyme, being present in the blood. Since lactase does not occur in the blood, any unsplit lactose introduced into the circulation as such is incapable of being utilized. This ready assimilability of maltose is occasionally mentioned as a point in favor of the use of maltose-dextrin, but we should not lose sight of two important facts in this connection. The first is the possibility of too rapid absorption being of doubtful value, and the second that the comparatively complex dextrins are present in almost as large amounts as the maltose.

From this brief review it is evident that the question as to the superiority of any one sugar over another for use in infant feeding is by no means definitely settled. During the summer of 1912, at the suggestion and under the direction of Dr. E. M. Colie, Jr., we had opportunity to use different sugars in feeding infants treated at the Seaside Hospital of St. John's Guild.

The infants observed were either convalescent from more or less severe gastroenteric disturbance (dyspepsia or intoxication), or were suffering from mild, more or less chronic conditions (chronic intestinal indigestion; "Bilanzstörung" or slight "Dyspepsie"). No normal cases were observed, nor were the cases "selected" for any particular sugar.

Dilutions of whole milk with sterile tap water or barley water (about 1 per cent. starch) constituted the food in the majority of instances. Top milk (7 or 10 per cent. fat) dilutions, whey, whey-cream mixtures, and casein milk were employed, but the inclusion of the cases receiving these latter foods complicates the tables without having any marked influence on the final results. The attempt has been made to give in the tables the points of greatest importance, but it is evident that accuracy has suffered in the process of condensation. The relation of fat and casein was always the same, and the percentage of fat was low, so that figures bearing on these points are omitted. In estimating the sugar percentage and the energy quotient, no attempt has been made to secure absolute accuracy. It was assumed that whole cow's milk contains 4.5 per cent. lactose, and that the addition of 1 ounce of sugar by weight to a total mixture of 100 ounces by volume, increased the sugar by 1 per cent. All calculations were based upon the amount of food ordered, and when the refusal of a portion occurred this was disregarded.

In addition, two other important sources of error must be considered. It is not uncommon to see two apparently normal infants react very differently to the same food, one gaining in a satisfactory manner, while the other fails to gain or suffers serious digestive disturbances. Therefore, it must be remembered that only by the observation of considerable numbers of cases can the error from individual peculiarities of the infants be so diminished as to be unimportant.

If one child is given a food containing 4 per cent. fat, 6 per cent. sugar and 1 per cent. protein, its progress could not be compared with that of a second receiving a food containing 1 per cent. fat, 6 per cent of another sugar, and 3.5 per cent. protein. In the cases reported, however, no such extreme variation in the composition of the food occurred, and they were so grouped that the variations in food offset each other. However, in view of the limited number of cases reported, it must be understood that the evidence presented is by no means conclusive, merely suggestive.

The sugars used were:—

Commercial lactose
Commercial saccharose
Pure maltose
80% maltose { Maltose, 80%; Dextrin, 20%
Dextri-maltose { Maltose, 51%; Dextrin, 47%; Sodium chloride, 2%
*Malt soup extract { Maltose, 57.57-58.91%; Dextrin, 15.42-15.70%

## TABLE I.

## LACTOSE.

### A. Whole Milk Diluted with Water.

| Name | Total Sugar Per cent. | Energy Quotient | Weekly Weight Change in Grams | General Condition | Stools |
|---|---|---|---|---|---|
| Rich | 3.75 | 112 | —142 | Same | Remain dyspeptic |
| Romoli | 4 25 | 137 | +471 | Improved | Remain normal |
| Englehart | 4.25 | 100 | + 84 | Improved | Remain dyspeptic |
| Goldstein | 4.50 | 128 | + 99 | Same | Remain normal |
| Buckley, 1 | 4 50 | 101 | + 88 | Same | Remain normal |
| Crowe | 4 50 | 136 | + 28 | Worse | Worse |
| Bargolla | 5 00 | 122 | +248 | Same | Worse |
| Costello | 5 50 | 176 | 0 | Same | Worse |
| Empanella, 1 | 6 00 | 134 | —154 | Worse | Worse |
| Empanella, 2 | 6.00 | 173 | —357 | Same | Worse |
| Buckley, 2 | 6.00 | 115 | + 66 | Same | Remain dyspeptic |
| Shade | 6.00 | 106 | — 26 | Worse | Worse |
| Summerville | 6.00 | 118 | +231 | Same | Remain dyspeptic |
| Rossello, 1 | 6 00 | 167 | + 30 | Same | Improved |
| Barron, 1 | 6.00 | 82 | —110 | Same | Improved |
| Barron, 2 | 6.00 | 124 | +463 | Improved | Improved |
| Barron, 3 | 6 00 | 146 | —520 | Worse | Worse |
| Hensley | 6.00 | 136 | + 25 | Same | Remain normal |
| Heisler, 1 | 6.00 | 104 | —198 | Worse | Worse |
| Heisler, 2 | 6.00 | 100 | + 64 | Improved | Improved |
| Westphal | 6 00 | 128 | + 10 | Same | Remain dyspeptic |
| Rossello, 2 | 6.00 | 210 | —377 | Worse | Worse |
| Finnegan, 1 | 6 00 | 109 | +265 | Improved | Improved |
| Finnegan, 2 | 6.00 | 106 | — 44 | Same | Remain normal |
| Corina | 6 00 | 118 | —794 | Same | Improved |
| Rosello, 3 | 6 00 | 169 | +419 | Improved | Improved |
| | | | Gained 15—57.69%<br>Same 1— 3.84%<br>Lost 10—38.45% | Impr'v'd 6—23.07%<br>Same 14—53.8%<br>Worse 6—23.07% | Improved 7—26.9%<br>Same 10—38.4%<br>Worse 9—34.7% |
| Average | 5.55 | 129 | —131 | | |

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*The composition of the malt soup extract is not definitely fixed. The manufacturers say that each lot is made in the same manner and no considerable variations should occur. The manufacturers of Dextri-maltose, on the contrary, standardize their product.

## B. Whole Milk Diluted with Barley Water.

| | | | | | |
|---|---|---|---|---|---|
| Rosello | 2.25 | 143 | + 87 | Improved | Improved |
| White | 4.50 | 96 | —418 | Worse | Worse |
| Westphal | 4.50 | 109 | —165 | Worse | Worse |
| Mullen | 4.50 | 41 | —397 | Improved | Improved |
| Crawford | 4.50 | 115 | —595 | Same | Remain dyspeptic |
| Corina, 1 | 5.50 | 149 | +497 | Improved | Improved |
| Corina, 2 | 5.50 | 134 | — 28 | Same | Remain normal |
| | | | Gained 2—28.57%<br>Lost 5—71.43% | Improved 3—42.8%<br>Same 2—28.57%<br>Worse 2—28.57% | Improved 3—42.8%<br>Same 2—28.57%<br>Worse 2—28.57% |
| Average | 4.46 | 112 | —145 | | |

## TABLE II.

### SACCHAROSE

### Whole Milk Diluted with Barley Water.

| Name | Lactose Per cent. | Total Sugar Per cent | Energy Quotient | Weekly Weight Change in Grams | General Condition | Stools |
|---|---|---|---|---|---|---|
| Corina, 1 | 3.40 | 5.00 | 101 | —529 | Same | Worse |
| Corina, 2 | 3.40 | 5.00 | 98 | +397 | Same | Remain normal |
| Miller, M | 2.25 | 6 00 | 197 | — 65 | Same | Remain normal |
| Miller, A | 2.25 | 6.00 | 164 | — 99 | Same | Remain dyspeptic |
| Hensley | 5.30 | 7.30 | 182 | +198 | Same | Worse |
| | | | | | Improved 0<br>Same 5 100%<br>Worse 0 | Improved 0<br>Same 3—60%<br>Worse 2—40% |
| Average | 3.32 | 5.86 | 148 | — 98 | | |

## TABLE III.

### PURE MALTOSE.

### A. Whole Milk Diluted with Water.

| Name | Lactose Per cent | Total Sugar Per cent | Energy Quotient | Weekly Wt. Change in Grams | General Condition | Stools |
|---|---|---|---|---|---|---|
| Empanella, 1 | 1.30 | 3.80 | 180 | 0 | Same | Remain Constipated |
| Empanella, 2 | 2.70 | 7.70 | 236 | +536 | Improved | Improved |
| Solomon | 2.25 | 6.25 | 151 | — 57 | Same | Remain Constipated |
| | | | | Gained 1—33.3%<br>Same 1—33.3%<br>Lost 1—33.3% | Improved 1—33.3%<br>Same 2—66.6<br>Worse 0 | Improved 1—33 3%<br>Same 2—66.6<br>Worse 0 |
| Average | 2.08 | 5.91 | 189 | +153 | | |

### B. Whole Milk Diluted with Barley Water.

| | | | | | | |
|---|---|---|---|---|---|---|
| Goldstein, 1 | 2.25 | 4.00 | 1.61 | 0 | Same | Remain Constipated |
| Goldstein, 2 | 2.25 | 5.30 | 80 | + 50 | Same | Remain Constipated |
| | | | | Gained 1—50%<br>Same 1—50% | Improved 0<br>Same 2—100%<br>Worse 0 | Improved 0<br>Same 2—100%<br>Worse 0 |
| Average | 2.25 | 4.65 | 70 | + 25 | | |

## TABLE IV.

### 80% MALTOSE.

### A. Whole Milk Diluted with Water.

| Name | Lactose Per cent. | Total Sugar Per cent | Energy Quotient | Weekly Weight Change in Grams | General Condition | Stools |
|---|---|---|---|---|---|---|
| Finnegan...... | 2.25 | 3.75 | 143 | −19 | Worse | Worse |
| Rich........... | 2.25 | 3.75 | 118 | + 6.6 | Worse | Worse |
| Goldstein...... | 2.25 | 4.25 | 130 | 0 | Same | Remain constipated |
| Stessel ........ | 2.25 | 4.25 | 140 | + 1.61 | Improved | Remain normal |
| Bargolla ...... | 3.00 | 5.00 | 122 | +57 | Same | Worse |
| | | | | Gained 3—60%<br>Same 1—20%<br>Lost 1—20% | Improved 1—20%<br>Same 2—40%<br>Worse 2—40% | Improved 0<br>Same 2—40%<br>Worse 3—60% |
| Average ...... | 2.40 | 4.20 | 131 | +41 | | |

### B. Whole Milk Diluted with Barley Water.

| Name | Lactose Per cent. | Total Sugar Per cent | Energy Quotient | Weekly Weight Change in Grams | General Condition | Stools |
|---|---|---|---|---|---|---|
| Crawford...... | 2,25 | 3.80 | 100 | — 80 | Same | Remain normal |
| Fisces.......... | 3.90 | 6.00 | 138 | +368 | Same | Worse |
| | | | | Gained 1—50%<br>Lost 1—50% | Improved 0<br>Same 2—100%<br>Worse 0 | Improved 0<br>Same 1—50%<br>Worse 1—50% |
| Average....... | 3.07 | 4.90 | 119 | +144 | | |

## TABLE V.

### DEXTRI-MALTOSE

### A. Whole Milk Diluted with Water.

| Name | Lactose Per cent. | Total Sugar Per cent. | Energy Quotient | Weekly Weight Change in Grams | General Condition | Stools |
|---|---|---|---|---|---|---|
| Costello........ | 1.70 | 3.20 | 126 | 0 | Same | Worse |
| Romoli......... | 2.25 | 4.00 | 130 | — 42 | Same | Worse |
| Hensley........ | 1.60 | 6.00 | 120 | +397 | Same | Remain normal |
| Empanella.... | 1.80 | 6.80 | 192 | +331 | Worse | Worse |
| O'Malley...... | 2.00 | 7.00 | 168 | +227 | Improved | Remain dyspeptic |
| Galato, 1....... | 6.00 | 11.00 | 269 | — 20 | Same | Worse |
| Galato, 2....... | 6.00 | 11.00 | 279 | +139 | Improved | Improved |
| | | | | Gained 4—57.14%<br>Same 1—14.29%<br>Lost 2—28.57% | Imp. 2—28.57%<br>Same 4—57.14%<br>Worse 1—14.28% | Imp. 1—14.28%<br>Same 2—28.57%<br>Worse 4—57.14% |
| Average....... | 3.05 | 7.00 | 183 | +147 | | |

### B. Whole Milk Diluted with Barley Water.

| Name | Lactose Per cent. | Total Sugar Per cent. | Energy Quotient | Weekly Weight Change in Grams | General Condition | Stools |
|---|---|---|---|---|---|---|
| Goldstein ..... | 2.25 | 3.25 | 141 | — 49 | Same | Remain Constipated |
| Koch .......... | 1.50 | 3.50 | 119 | —178 | Same | Remaid normal |
| Crawford ..... | 2.25 | 3.75 | 107 | + 99 | Improved | Remain normal |
| Schmidt....... | 2 25 | 3.80 | 118 | —207 | Same | Remain normal |
| Miller, M...... | 6.00 | 7.60 | 195 | — 37 | Worse | Worse |
| | | | | Gained 1—20%<br>Same 0<br>Lost 4—80% | Imp. 1—20%<br>Same 3—60%<br>Worse 1—20% | Imp. 0<br>Same 4—80%<br>Worse 1—20% |
| Average ...... | 2.85 | 4.38 | 136 | —372 | | |

## TABLE VI.

### MALT-SOUP EXTRACT.

### A. Whole Milk Diluted with Water.

| Name | Lactose Per cent. | Total Sugar Per cent. | Energy Quotient | Weekly Weight Change in Grams | General Condition | Stools |
|---|---|---|---|---|---|---|
| Rosello, 1 | 2.25 | 3.25 | 147 | +694 | Improved | Remain normal |
| Logan | 2.25 | 4.25 | 75 | +496 | Improved | Remain dyspeptic |
| McCarthy | 6.00 | 7.00 | 204 | +172 | Improved | Remain normal |
| Heisler | 6.00 | 7.00 | 186 | +112 | Same | Become dyspeptic |
| Rosello, 2 | 6.00 | 8.40 | 188 | + 79 | Improved | Remain same |
| Barron | 6.00 | 9.00 | 182 | −313 | Worse | Become dyspeptic |
| Summerville | 6.00 | 9.00 | 144 | — 71 | Worse | Become worse |
| Barron | 6.00 | 9.00 | 230 | +213 | Improved | Improved |
| Schosi | 6.00 | 10.00 | 193 | +164 | Improved | Remain normal |
| Schosi | 6.00 | 10.00 | 199 | + 79 | Same | Remain normal |
| | | | | Gained 8—80%<br>Same 0<br>Lost 2—20% | Improved 6—60%<br>Same 2—20%<br>Worse 2—20% | Improved 1—10%<br>Same 6—60%<br>Worse 3—30% |
| Average | 5.25 | 7.69 | 175 | +162 | | |

### B. Whole Milk Diluted with Barley Water.

| | | | | | | |
|---|---|---|---|---|---|---|
| Hitchings | 1.90 | 4.00 | 170 | −174 | Worse | Same |
| Stessel, 1 | 2.25 | 4.25 | 141 | + 79 | Improved | Improved |
| Galato | 2.25 | 4.75 | 179 | + 99 | Improved | Remain normal |
| Stessel, 2 | 3.40 | 7.40 | 226 | +694 | Worse | Become dyspeptic |
| Miller, A | 6.00 | 7.60 | 202 | + 21 | Improved | Remain dyspeptic |
| Mullen | 6.00 | 8.00 | 89 | +263 | Improved | Remain normal |
| Barron | 6.00 | 9.00 | 172 | +127 | Worse | Remain dyspeptic |
| | | | | Gained 6—85.72%<br>Same 0<br>Lost 1—14.28% | Imp. 4—57.14%<br>Same 0<br>Worse 3—42.85% | Improved 1—14.28%<br>Same 5—28.56%<br>Worse 1—14.28% |
| Average | 3.97 | 6.42 | 168 | +158 | | |

If the results secured by the addition of lactose to the milk modifications are examined, it is seen that the use of a cereal diluent apparently has a favorable influence if we judge by the character of the stools and the general condition. If, however, the weight change be taken as criterion, it is evident that the reverse is true. It should be noted, however, that the percentage of sugar and the energy quotient are lower for the barley water series than they are for that in which plain water was used as diluent.

Where 80 per cent. maltose was used, there were only 2 cases in which a cereal diluent was employed. So far as the limited number of cases allows a judgment, no appreciable difference existed between the two series. The same may be said in regard to the pure maltose, but in the dextri-maltose series a greater gain occurred where plain water was used as a diluent, but the nature of the stools also became worse in a larger number than in those cases where a cereal diluent was used. It is seen, however, that the sugar percentage was quite different in the two series, which might account for both facts. Similar conditions exist in regard to malt extract and the same explanation may be offered.

Apparently the use of a cereal diluent did not exert any very marked influence. Unfortunately, in the saccharose series a cereal diluent was used throughout, so no comparison is available, but assuming that the results would have been the same here as with the other sugars, we may assemble the averages in the following table, grouping together all cases fed any particular sugar, irrespective of the diluent used.

| | Total Sugar Per cent. | Energy Quotient | Weekly Weight Change in Grams | General Condition | Stools |
|---|---|---|---|---|---|
| Lactose ....... | 5.00 | 120 | −138<br>Gained 17—51 5%<br>Same 1— 3.9%<br>Lost 15—45.4% | Impr'v'd 9—27.27%<br>Same 16—48.42%<br>Worse 8—24 24% | Improved 10—30 30%<br>Same 12—36.36%<br>Worse 11—33.33% |
| Saccharose.... | 5.86 | 148 | −98<br>Gained 2—40%<br>Same 0<br>Lost 3—60% | Improved 0<br>Same 5—100%<br>Worse 0 | Improved 0<br>Same 3—60%<br>Worse 2—40% |
| 80% Maltose... | 4.55 | 125 | +92.5<br>Gained 4—57.14%<br>Same 1—14.28%<br>Lost 2—28.57% | Impr'v'd 1—14.25%<br>Same 4—57.14%<br>Worse 2—28.57% | Improved 0<br>Same 3—42 86%<br>Worse 4—57.14% |
| Maltose........ | 5.28 | 179 | +89<br>Gained 2—40%<br>Same 2—40%<br>Lost 1—20% | Improved 1—20%<br>Same 4—80%<br>Worse 0 | Improved 1—20%<br>Same 4—80%<br>Worse 0 |
| DextriMaltose | 5.69 | 159 | −112.5<br>Gained 5—41.66%<br>Same 1—8.33%<br>Lost 6—50.00% | Impr'v'd 3—25%<br>Same 7—58.33%<br>Worse 2—16.66% | Improved 1— 8.33%<br>Same 6—50.00%<br>Worse 5—41.66% |
| Malt Soup Extract | 7.05 | 171 | +144<br>Gained 14—82.55%<br>Same 0<br>Lost 3—17.65% | Imp. 10—58.82%<br>Same 2—11.76%<br>Worse 5—29.41% | Improved 2—11.76%<br>Same 11—64.70%<br>Worse 4—23 52% |

So far as the number of cases justifies, certain fairly definite conclusions can be drawn from the results collected in the last table.

One of the most important indications of an infant's satisfactory progress is the weight increase. From the last table it is seen that the greatest sum total weight increase occurred in the series of infants fed on mixtures containing malt-soup extract, while the greatest total loss occurred in the lactose-fed series. An examination of the individual tables shows, however, that some of the infants receiving lactose suffered considerable losses, more than offsetting the moderate gains in a larger number of cases. It is probably fairer to rate the sugars according to the percentage of cases which showed an increase in weight.

Mere increase in weight alone is not a reliable index of progress

When, however, the state of the stools is good and the infant's general condition satisfactory, more trustworthy conclusions may be drawn. If these three factors are considered, the following grouping is possible:

| | Gained in Weight | General Condition Improved | Stools became Worse |
|---|---|---|---|
| Malt-soup extract . . . | 82.35% | 58.82% | 23.52% |
| Lactose . . . . . . . . . | 51.50% | 27.27% | 33.33% |
| Dextri-maltose . . . . . | 41.66% | 25.00% | 41.66% |

Thus, it is evident that of the three sugars considered, malt-soup extract was distinctly superior in all three respects, while dextri-maltose is decidedly of less value than lactose. Pure maltose, saccharose, and 80 per cent. maltose cannot be included in the table, for 80 per cent. maltose caused a weight increase superior to that present in the lactose series, but the general condition was improved in less than 15 per cent. of the cases receiving the former sugar and the stools became worse in 57 per cent. of them. Slightly similar conditions exist in regard to pure maltose and saccharose. This is due, in all probability, to the small number of cases studied, so we may dismiss the three latter sugars from consideration until more evidence is forthcoming.

Why should malt-soup extract produce better results than those following the use of the other sugars?

The first possibility is that many of our infants had received sugar injury before coming under observation; and, as has been mentioned, lactose and saccharose are much more used in America, by the laity certainly, in feeding infants. Thus, it is easily understood why a malt sugar would be better utilized than lactose. But the effects following the use of dextri-maltose compel us to discard this explanation as untenable.

It might be supposed that the presence of an alkali in the malt extract exerted an influence, but many of the infants fed upon other sugars received 5, 10 or 15 per cent. lime water.

The protein content of the malt extract present in the milk dilution is so small that it could scarcely be of importance in cases where the milk protein was always high.

The quantity as well as the quality of the food exerts an influence on the weight change. Reference to Table VII. shows that the sugar percentage and the caloric value of the malt soup extract series are higher than in either lactose or dextri-maltose series. This would seem to offer a satisfactory explanation for the weight increase after malt extract, but why did not the dextri-maltose series show to better advantage than did the series receiving lactose?

Moreover, the general condition and the stools in the malt-extract series are better than in the other two series, which is not compatible with over-feeding.

In conclusion, it must be clearly understood that the evidence presented has no bearing upon the selection of a sugar for feeding "normal" infants. I have seen nothing in the literature to prove nor have we made any observations in support of the contention that the maltose-dextrin mixtures are in any way superior to lactose for addition to the food of the average sound infant. That malt-soup extract is superior to lactose and the latter in turn preferable to dextri-maltose seems indicated by our results, but the small number of cases and the limited time of observation make one hesitate to draw positive conclusions.

It is with pleasure that I acknowledge my obligation to Dr. Colie for the oppportunity of reporting our results and also to my associates, Dr. E. J. Healey and Dr. F. H. Carber, who kindly placed at my disposal records of cases under their charge.

***Department of Experimental Medicine.***

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# THE DETERIORATION OF PHARMACEUTICAL PREPARATIONS.

FRANK R. ELDRED

Address of the Chairman of the Scientific Section, American Pharmaceutical Association, Nashville, Tenn., Aug. 18-23, 1913, Reprinted from the September Number of the Jour. Am. Pharm. Assoc., Vol. II, p. 1109.

The standardization of galenical preparations is such a well-worn topic that I hesitate to refer to it in this connection, yet it is the logical starting point in the consideration of their stability.

The adoption of standards for these products marks the most important advance which has been made in their manufacture. Before such standards became possible many years had been devoted to the analysis of drugs and later to the development of assay methods for determining the active principles which had been found. It is almost needless to say that an apparently unlimited amount of work yet remains to be done in both these fields. Our knowledge of the chemistry of many important drugs is so slight that we cannot even attempt to develop chemical methods for their standardization; other drugs whose chemistry is better understood cannot be accurately assayed because suitable methods for this purpose have not been devised and moreover, when we examine the assay processes which are in general use we find many imperfections. Steady progress has, however, been made in the development of methods and apparatus for assaying drugs and today the manufacturer may find that he is forced to use a process which he knows to be inaccurate because it was perhaps the best available when the Pharmacopœia gave it a legal standing. It is obvious that standards could not be established until fairly satisfactory assay processes had been agreed upon. The immediate effect of such standards was much greater uniformity in the strength of preparations standardized, for although the methods were not perfect and results not always as accurate as they should have been, yet the first serious attempt was being made to bring about uniformity and variations in strength became almost negligable.

After certain standards and methods had received official recognition the question of the stability of the standardized products began to receive attention._ Previous to this time chemists engaged in this line of work had been so busy developing the assay methods, without which, deterioration studies could not be made, that no systematic investigation of the changes which might occur in such standardized products after their manufacture, had been undertaken. Many persons, forgetting the years which had been consumed in acquiring our present knowledge of drugs, seemed to think that it would be a simple matter to determine once for all the degree of stability of these preparations and investigations and discussions of this matter became the fashion. So much has been said and written that it was thought desirable to summarize our knowledge of the subject but a review of the literature, so full of contradictory "conclusions," seems to render this impossible except in the most general way. Even the results of investigations in many cases show the widest variance, for instance one chemist finds that some lots of fluid extract and tincture of cinchona show deterioration, while other chemists who have examined many lots of these preparations have found no deterioration. The occasional precipitation of alkaloids from cinchona preparations may be due to differences in the drug used, or to the conditions of manufacture and storage and only a careful study will determine the cause of this deterioration so that steps may be taken to prevent it. It is safe to say that in more than one laboratory, studies are being made with this end in view. We may confidently expect that whenever products are found to deteriorate every effort will be made to overcome this difficulty, and the fact that most of these preparations are so stable encourages the belief that in nearly all cases deterioration can be practically eliminated. Fluid extract of coca which is fortunately of little importance is the only fluid extract which has been uniformly found to deteriorate. Indeed the only general conclusion which can be drawn after a careful consideration of the literature is, that as a class galenical preparations are surprisingly stable. It is extremely unfortunate that a few writers acting as alarmists have tried to show that the reverse is true and I wish to call attention to some of their statements. We have been told that fluid extract of ergot is worthless after it is six months old, yet the clinical reputation of ergot in this country was probably largely established by the use of a fluid extract which the manufacturer aged for one year before bottling. The most careful studies of this preparation have failed to show any loss of strength in two years and it is difficult to say at this time how much longer it can be kept without deterioration as this can only be definitely determined by further aging tests. Very old samples of the crude drug have also been shown to be of average activity despite the more or less prevalent opinion that such drug is worthless. Again a recent writer in one of the pharmaceutical journals making use of the data of various investigators has arrived at some conclusions differing materially from those of the original authors and in no way justified by the data presented. This writer says that fluid extracts of ergot and digitalis should not be relied upon when over six months old although no evidence whatever is offered in support of this statement. He also states that physicians should discard "as far as possible" all fluid extracts which are over two years old, while the data presented could in no way lead to this conclusion. Some manufacturers also, have made capital of reputed deterioration to call attention to special means of preserving drugs from influences which have not definitely been shown to affect them. For instance patents have been issued on a process for destroying the hydrolytic enzymes which cause the deterioration of digitalis preparations, and other special methods for preserving crude digitalis and its preparations have been exploited; yet Hatcher has recently shown that fluid extracts at least twenty years old show no evidences of deterioration, while the crude drug preserved with ordinary care has been proven to be perfectly stable thus rendering unnecessary any of the special methods of drying or preservation. Although Hatcher's results may not be taken as final, his careful work certainly shows how ridiculous it is to try to explain the cause or devise means for preventing deterioration which is not known to take place. This brings us back to the consideration of accuracy in methods of valuing drugs, for other investigators, using methods different from that employed by Hatcher, have fixed upon various rates of deterioration for fluid extracts and tinctures of digitalis. I have reminded you of our scant knowledge of the chemistry of many important drugs and of our imperfect chemical assay methods and these statements apply also, perhaps in less degree, to our knowledge of the pharmacology of many such drugs and in an even greater degree to the physiological assay methods. While we are attempting to study the deterioration of such drugs as ergot and digitalis by diverse methods upon which pharmacologists are not yet in accord we must not therefore allow ourselves to draw too definite conclusions. If conservative in this respect we can acquire much useful information from these studies in regard to the methods used as well as to the stability of the preparations. We cannot expect the same degree of accuracy in physiological methods that has been attained in chemical methods on account of the unavoidable variations in the animals used and also from the fact that it is a comparatively short time since physiological methods were first used for the standardization of drugs. No single method should be relied upon and results upon only a few lots should not be accepted, as evidenced by the results on fluid extract of cinchona which have been cited. With these facts in mind an impartial study of the literature can lead only to the conclusion that there is no positive proof, with one or two important exceptions, that galenicals deteriorate in any serious degree.

Such important advances have been made in the manufacture of galenicals that the members of the pharmaceutical profession may well congratulate themselves upon this fact instead of agreeing with one of our friends who, upon the basis of his studies of ergot deterioration has depicted the situation as "horrible." I would by no means have you infer that I think we should be satisfied, for on the other hand I believe that it is the duty of anyone engaged in the manufacture of medicinal preparations to strive with all the means at his disposal to improve both preparations and methods for testing them.

It has recently been very popular to advocate the dating of pharmaceutical products, and certainly no one will deny that this should be done whenever our knowledge is sufficiently exact to establish a period after which the preparation should not be used. Until this can be done nothing could be more illogical than to date such preparations. For example it has frequently been urged that digitalis preparations should be dated, yet Hatcher's work seems to have established the fact that these preparations will keep twenty or thirty years; what can be accomplished then by dating them? Surely preparations older than this will not be frequently used! If the dates of manufacture are placed upon preparations when no definite knowledge in regard to their keeping qualities is available who shall decide when these preparations must be discarded? One writer who advocates placing the date of manufacture on certain preparations says that while it is very difficult to set a definite time limit on a preparation that deteriorates; yet the dating of such preparations enables the pharmacist to use his expert judgment as to whether or not a preparation is too old to be used. If this knowledge is not available to the manufacturer where can we expect the dispenser to obtain the information? The answer is clear, the fact of deterioration must be determined from the preparation itself and not from any date appearing on the label. The fact that where deterioration occurs, different lots will deteriorate at very different rates, cannot be too strongly emphasized; this is due no doubt to differences in the preparation itself as well as to conditions of storage. If galenical preparations are not protected from extremes of temperature and from direct sunlight, and are not stored in well stoppered amber bottles more rapid changes must be looked for in the preparations. Differences in various lots of the same preparation and even in different bottles of the same lot are seen in a marked degree in solution of hydrogen dioxide, so that any time limit which could be established would, therefore, have the disadvantage of being too near in some cases and too remote in others, and the same condition will be found to exist with other preparations which deteriorate. It might be urged, that until we can absolutely demonstrate that a preparation does not deteriorate at all during a long period of years, we should give the consumer the benefit of the doubt and require the manufacturer to date his packages, but the fallacy of this argument is easily seen when it is considered that most of our information points to the great stability of such products and that if the pharmacist was arbitrarily required to discard preparations of a certain age the consumer would ultimately have to pay for this uncalled for waste. The careful pharmacist will in any event turn over his stock of pharmaceuticals as rapidly as possible both for the purpose of keeping his stock fresh and to increase his financial gains; in this he is certainly to be encouraged.

Although there can be no doubt that deteriorated products have been dispensed and will be dispensed in the future, conditions are rapidly improving and, indeed, have never been as serious as many writers would lead us to believe. It is undoubtedly true also, that improvements in manufacturing processes will keep pace with improvements in analytical and pharmacological methods, so that as fast as the fact of deterioration can be established, steps will be taken to overcome the difficulty. Let us not forget that even though methods are imperfect and preparations may occasionally deteriorate the resulting variations in strength will in most cases be much less than the variations which would occur in unstandardized preparations. We are therefore making good progress and a little patience only is required until the bugaboo of deterioration will be, in one way or another, overcome.

*Scientific Division.*

# THE ASSAY OF INDIVIDUAL PLANTS OF DATURA STRAMONIUM L., DATURA TATULA L. AND OTHER SPECIES AND VARIETIES

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[Presented to the Pharmaceutical Section of the American Chemical Society at the meeting held in Milwaukee, March, 1913, and reprinted from the Journal of Industrial and Engineering Chemistry, Vol. 5, Page 1014.]

It has been stated in a recent communication,[1] (8th, Int. Cong. App. Chemistry, vol. 17, p. 57) that the examination of individual plants of the daturas for their total alkaloidal content has been undertaken as a means of following the effects of prolonged cultivation upon the percentage of alkaloids, and as a means of selecting high yielding individual plants for breeding purposes. The present discussion is a continuation of this investigation, together with additional data upon other species and varieties not heretofore included. A review of the literature on stramonium was given in the original communication. This was followed by the assays of several individual plants of Datura stramonium L. and Datura tatula L. Seeds were collected from all the assayed plants and those from the individuals containing the highest and lowest percentages of alkaloids from each of the two species were planted May 30th, 1912. The results obtained from these plantings are considered in the present discussion.

The conditions of growth were practically the same as those under which the parent plants were produced. The soil, a stiff clay loam though not identical, was that of an adjacent field and of a similar physical character. The plot was fairly uniform except for an excess of sand and gravel at one end. The plants growing at this end were smaller and less vigorous than the others. When about three inches high they were thinned to four feet in the row. Cultivation was begun at this time and continued throughout the growing season.

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[1] See also Lilly Scientific Bulletin Ser. 1, No. 3, page 108.

Eight plantings were made, four of Datura stramonium L. and four of Datura tatula L. These were made from the highest and lowest yielding individuals from each of the two species, as given in the original communication. The plants indicating these extremes, assayed 0.55% and 0.46% in Datura stramonium L. and 0.65% and 0.47% in Datura tatula L. Four of the above plots received an application of normal commercial fertilizer at the rate of six hundred pounds per acre. This was applied when the plants were about one foot high.

The collection of samples of leaves for assay from individual plants of each of the eight plots was made early in September. These samples were dried at room temperature, granulated and sealed in amber bottles until assayed. Following are the results obtained from nineteen selections of individual plants from Datura stramonium L. Ten of these were from the high and nine from the low yielding parent.

TABLE No. 1—*Datura Stramonium L.*

Highest plant originally assayed 0.55 per cent.

| Without Fertilizer | | | With Fertilizer | | |
|---|---|---|---|---|---|
| No. | Source | Assay Per cent. | No. | Source | Assay Per cent. |
| B-980 | Parent plant | 0.55 | B-980 | Parent plant | 0.55 |
| B-1349 | Selection | 0.50 | A-1360 | Selection | 0.67 |
| B-1351 | " | 0.56 | B-1361 | " | 0.66 |
| B-1352 | " | 0.50 | B-1362 | " | 0.62 |
| B-1353 | " | 0.54 | B-1363 | " | 0.65 |
| B-1354 | " | 0.42 | B-1364 | " | 0.48 |
| | Average | 0.50 | | | 0.61 |

Lowest plant originally assayed 0.46 per cent.

| Without Fertilizer | | | With Fertilizer | | |
|---|---|---|---|---|---|
| No. | Source | Assay Per cent. | No. | Source | Assay Per cent. |
| B-982 | Parent plant | 0.46 | B-982 | Parent plant | 0.46 |
| B-1355 | Selection | 0.58 | B-1357 | Selection | 0.64 |
| B-1356 | " | 0.57 | B-1368 | " | 0.68 |
| B-1365 | " | 0.58 | B-1369 | " | 0.58 |
| B-1366 | " | 0.65 | B-1370 | " | 0.71 |
| B-1367 | " | 0.63 | | | |
| | Average | 0.60 | | Average | 0.64 |

The *Datura tatula* L. selections of which there were ten each from the high and low yielding parents, gave the following results:

TABLE No. II—*Datura Tatula L.*

Highest plant originally assayed 0.65 per cent.

| Without Fertilizer | | | With Fertilizer | | |
|---|---|---|---|---|---|
| No. | Source | Assay Per cent. | No. | Source | Assay Per cent. |
| B-984 | Parent plant | 0.65 | B-984 | Parent plant | 0.65 |
| B-1344 | Selection | 0.53 | B-1378 | Selection | 0.72 |
| B-1348 | " | 0.56 | B-1379 | " | 0.75 |
| B-1376 | " | 0.77 | B-1380 | " | 0.69 |
| B-1377 | " | 0.59 | B-1381 | " | 0.73 |
| B-1383 | " | 0.64 | B-1382 | " | 0.44 |
| Average | | 0.62 | Average | | 0.68 |

Lowest plant originally assayed 0.47 per cent.

| Without Fertilizer | | | With Fertilizer | | |
|---|---|---|---|---|---|
| No. | Source | Assay Per cent. | No. | Source | Assay Per cent. |
| B-985 | Parent plant | 0.47 | B-985 | Parent plant | 0.47 |
| B-1341 | Selection | 0.47 | B-1371 | Selection | 0.66 |
| B-1342 | " | 0.45 | B-1372 | " | 0.50 |
| B-1343 | " | 0.52 | B-1373 | " | 0.48 |
| B-1346 | " | 0.45 | B-1374 | " | 0.53 |
| B-1347 | " | 0.57 | B-1375 | " | 0.54 |
| Average | | 0.49 | Average | | 0.54 |

A small plot of Datura ferox L. was grown from seed obtained from Haag & Schmidt, of Erfurt, Germany. Four selections were made and samples of leaves from these selected plants assayed as follows:

TABLE No. III

| No. | Source | Assay |
|---|---|---|
| B-1345 | Datura ferox | 0.53% |
| B-1350 | " | 0.62% |
| B-1358 | " | 0.70% |
| B-1359 | " | 0.66% |

A small planting of Datura humulus flava was made of seed from the above source. A mixed sample of leaves assayed 0.42%. Individual selections could not be made on account of the failure of the plants to produce seed. This was due to the late planting and injury from early frost. The plants did not flower until September and were extremely sensitive to frost.

The foregoing figures indicate a considerable range of variations in the percentage of alkaloids as found in individual plants. This variation in the progeny of both the Datura stramonium L. and Datura tatula L. selections is greater than that of the two parent plants. In the species stramonium this is 0.09% in the parents, while in the progeny it is 0.23% for the selections from both the fertilized and non-fertilized plots. The highest yielding plant was produced by the low parent upon a fertilized plot while the lowest yielding plant was produced by the high parent upon a non-fertilized plot. Contrary to expectations the higher averages were obtained both upon the fertilized and non-fertilized plots from the low yielding parent. For the fertilized plots these averages are 0.64% from the low parent and 0.61% from the high parent. For the non-fertilized plots they are 0.60% from the low parent and 0.50% from the high parent.

In the species tatula the variation of the two parent plants is 0.18% while in the progeny it is 0.31% and 0.32% upon the fertilized and non-fertilized plots respectively. The highest yielding plant was produced by the high parent upon a non-fertilized plot, while the lowest yielding plant was also produced by the high yielding parent but upon a fertilized plot. The latter was an abnormal plant producing few leaves and for this reason might be expected to give a low yield. The next lowest appears, according to expectation, in the progeny of the low parent and upon a non-fertilized plot. The averages in this case are the reverse of what was noted for the species stramonium and are according to expectations. The higher averages are obtained from the progeny of the high parent and from the fertilized plots. For the fertilized plots these averages are 0.68% from the high parent and 0.54% from the low parent. For the non-fertilized plots they are 0.62% from the high parent and 0.49% from the low parent.

The following table shows extremes as noted in parents and offsprings and the variations as found upon fertilized and non-fertilized soils.

TABLE No. IV—*Datura Stramonium.*

| | Assay, Highest Yielding Plant | Assay, Lowest Yielding Plant | Variations |
|---|---|---|---|
| Parent plant | 0.55% | 0.46% | 0.09% |
| Non-fertilized | 0.65% | 0.42% | 0.23% |
| Fertilized | 0.71% | 0.48% | 0.23% |
| | *Datura Tatula* | | |
| Parent plant | 0.65% | 0.47% | 0.18% |
| Non-fertilized | 0.77% | 0.45% | 0.32% |
| Fertilized | 0.75% | 0.44% | 0.31% |

The greater range of variations in individual plant yield as found in these second generation plants is probably due to the fact that the parent plants represented mixed strains. Such mixed strains resulting from promiscuous pollination might produce plants giving altogether different yields from the parents. When the original selections were made no precautions were taken to prevent cross pollination. This precaution is necessary with the Daturas if known parentage is desired. It has not been conclusively shown that the property of a plant to produce a definite percentage of alkaloids, is uniformly transmitted to the offsprings. Inbreeding or close pollination of all selected plants is necessary in the study of the transmission of such a character. The results so far obtained indicate that the seeds from open pollinated plants are either of composite parentage or that the character under consideration is not uniformly inherited.

The results seem to point toward the former supposition with certain exceptions. Some degree of uniformity is to be noted in the assays of these second generation plants. There is more uniformity among the individuals within the groups assayed than there is between these individuals and the parent plants. Considering only the non-fertilized plots in this respect the progeny of the high yielding stramonium is most uniform. The parent assayed 0.55% and the progeny 0.50%, 0.56%, 0.50%, 0.54% and 0.42%. This uniformity within the groups is greatest in the tatula selections and is sufficient to maintain the averages in the order expected. Taking the average assay of the progeny of the low yielding parent from the non-fertilized plot as a unit, the averages from the other plots of this species follow in their expected order, i. e., 1.00 : 1.16 : 1.26 : 1.38. In the species stramonium, however, the uniformity within the groups is not sufficient to maintain this order. In this case the order is 1.00 : 1.10 : 0.82 1.01.

Greater extremes in the alkaloidal yield of the individual plants have been located than were found in the original investigations. This is doubtless due to the small number of plants originally assayed. It is believed that such extremes can be determined immediately by the examination of a sufficiently large number of individual plants produced under uniform conditions. Whether or not the greatest extremes in the two forms under discussion have been reached can only be determined by further study. The location of the highest extremes is the principal object. The possibility of the fixation of this high yielding property must then be determined by inbreeding. If such a procedure is possible, high yielding strains or races of the alkaloid producing plants can be developed for commercial purposes. With four exceptions all of the forty-three assayed plants have been inbred and mature seeds obtained from them for planting. Twenty crosses (hybridizations) have also been made between various individuals of the three species studied. A few reciprocal crosses have been made. The alkaloidal yield of these hybrid forms will be studied in comparison with the yield of the inbred parents and their progeny. Perfect uniformity in the behavior of any character cannot be expected in the first generation from inbred plants. This is due to latent tendencies, the elimination of which may require two or more generations by inbreeding.

It has already been stated that another object of this investigation was to follow the effects of prolonged cultivation upon the yield of alkaloids. The idea that cultivation causes a decrease in the value of medicinal plants has been current for many years, but apparently without foundation. The results obtained are contrary to this belief and are an indication of what may be accomplished through cultivation and improvement. All plants in the experimental plots have been under cultivation two years. The original source of the seed from which the cultivated strains were started is known absolutely in the case of Datura tatula L., as wild plants a few inches high were transplanted trom a vacant lot in Indianapolis to the experimental plot. Thus two years under cultivation is known to be correct for this species. This is not true however, with Datura stramonium L. The seeds from which the original planting was made were obtained in the London market and may or may not have been gathered from wild plants.

Mixed samples were collected for comparison from wild plants found growing adjacent to the experimental plots. Following is a table of the average assays of the wild and cultivated plants.

TABLE No.V—*Datura Stramonium*

Average Assays

| Source | 1910 | 1912<br>Fertilized | 1912<br>Non-fertilized |
|---|---|---|---|
| Original plants | 0.50% | | |
| High yielding plants | | 0.61% | 0.50% |
| Low yielding plants | | 0.64% | 0.60% |
| Wild plants | | | 0.28% |

*Datura Tatula*—Average Assays.

| Source | 1910 | 1912<br>Fertilized | 1912<br>Non-fertilized |
|---|---|---|---|
| Original plants | 0.58% | | |
| High yielding plants | | 0.68% | 0.62% |
| Low yielding plants | | 0.54% | 0.49% |
| Wild plants | | | 0.35% |

These averages indicate the beneficial effect of cultivation both with and without fertilizer. In most cases these averages are higher in the second year than in the first. The general averages of all the selected plants assayed this year is greater in each case than the averages of the plants originally assayed. The exceptions are most marked in the averages of the progeny from the low yielding parent. In Datura stramonium the averages are equal to or greater than the original average.

These forms will be continued under cultivation. Seed from the highest yielding individuals will again be planted and the resulting plants assayed in the same manner as those previously examined. In addition to the original objects of the investigation, i. e., the effects of cultivation and selection upon yield of alkaloids, the influence of hybridization upon this yield will also be noted. Other species and varieties will be included as seed supplies become available.

*Departments of Botany and Analytical Chemistry.*

SERIES 1 NUMBER 5

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# THE COMMERCIAL POSSIBILITIES IN GROWING MEDICINAL PLANTS.

F. A. MILLER, M. S.

[Presented to the Scientific Section of the American Pharmaceutical Association at the meeting held at Nashville, August, 1913, and reprinted from the Journal of the American Phar. Assoc. Vol. III, p. 34, Jan. 1914 ]

The commercial possibilities in growing medicinal plants are now recognized by the governments of England, Austria and the United States. The International Congress of Applied Chemistry, a society whose able efforts toward industrial development are now universally recognized, is taking active steps in the investigation of drug plant cultivation through an international committee. Universities, private institutions, and individuals have been induced to broaden their field of investigations to include medicinal plants.

From scattered, disconnected and poorly planned investigations of a minor character this work is gradually being organized with a determination that insures success. The dignity with which this movement is now being advanced removes all chances for doubt as to the practical value of drug growing. The success of such an undertaking will of necessity depend upon the commercial possibilities presented. The work in the United States has now reached a stage where these possibilities must be carefully considered.

The early history and evolution of the cultivation of medicinal plants within the U. S. and other countries has been treated elsewhere in an able manner by several authorities and need not be repeated here. It might be well to add, however, that most of the early work on drug growing was not exhaustive. It gave us little in the way of methods and in the behavior of medicinal plants under varied conditions. With the exception of a few forms such as cinchona, coca, and opium, little was accomplished upon commercial production and improvement. Of greater value to the progress of this work would be a careful interpretation of the progressive stages through which some of the valuable economic plants have passed in being brought to their present high state of development. Some of them are as old in cultivation as history itself. Still they are subject to improvement and, rich as some of them are in established varieties, other more valuable ones are being discovered and isolated which surpass their parents in many characteristics. Instead of attempting to solve the more practical phases of the question of drug growing as has been done in modern agriculture we have been busy suggesting long cumbersome lists of probable forms for cultivation and repeating antiquated methods of collecting, curing and packing which are of little practical value and have been handed down from early generations rather than evolved from actual investigations. The methods used have been in opposition to the principals of agriculture. No improved varieties of known cultural requirements were at hand and little effort has been made to produce them. The behavior of active principles under varied soil and climate conditions are imperfectly understood. Supplies of authentic seeds and plants of the more valuable forms are either unknown or extremely rare. And finally the greater number of requests for information upon the commercial possibilities of drug cultivation are from people not versed in the requirements of crude drugs. Not only are they deficient in this respect but they usually have a false impression as to the profits to be derived from such an undertaking. It is in this respect that the greatest care must be exercised in order that the interest now aroused may not be lost through an exaggeration of commercial possibilities. This has already been done to a certain extent and a portion of the recent literature dealing with this phase of drug growing invites discussion and some friendly criticism. The time is now current for the accumulation of practical data and its circulation under the most conservative and carefully guarded statements. There is a demand for information bearing directly upon immediate possibilities in drug growing. This demand should be met by information based upon actual practice and not upon theory and supposition. If this is impossible then a clear confession of our ignorance upon the subject should be in order.

The unfortunate error of one investigator in calculating the productivity of digitalis should serve as a caution to all those engaged in experimental drug growing. The estimated return of 4,606 dollars per acre was sufficiently attractive to engage the immediate attention of the daily press and of prominent trade journals. It created no little feeling and enthusiasm among florists by appearing in one of their most popular journals. This publication is noted for its practical information upon plant culture and is read almost exclusively by commercial growers. In view of the difficulties to be overcome in establishing drug growing it is unfortunate that information of this kind should find its way to practical men. The yield of 100 pounds produced upon 1,120 square feet as given by Prof. Newcomb does not differ greatly from that obtained by myself which was

101 pounds upon 1,089 square feet for Digitalis gloxinioides. This form is probably one of the heaviest producers of the purpurea strains and would not be representative for the official purpurea. The yield of 101 pounds was also obtained from 80 percent of the maximum number of plants which could be accommodated upon this size plot. This is probably a more nearly perfect stand than could be obtained upon a large scale where an acre or more was involved. That it would not be safe to calculate even the yield alone upon a basis of the above figures is indicated by the fact that only 288 pounds were obtained from one-fourth acre planted with a mixture of several varieties of the purpurea strains.

Kraemer in his fourth edition of Botany and Pharmacognosy includes a brief chapter of thirteen pages upon the cultivation of medicinal plants. However brief this chapter may appear there are over seven pages of the thirteen which are devoted strictly to plant names, both common and technical and including probably three hundred different species. The practical value of the publication of such a list in a general chapter upon drug cultivation is in doubt when we consider the very questionable virtue of many of the drugs mentioned. The great majority of these lesser important and questionable drugs are not only still abundant in the wild state but are marketed at extremely low prices and are used by pharmaceutical manufacturers in very small amounts. These conditions together with the growing tendency of the medical profession to reduce the number of drugs used would seem to justify the elimination of many of them from publications supposed to serve a practical purpose. It is said that the average practitioner will use less than twenty vegetable drugs. A few who may be called exceptions and those not of the modern school of medicine may use as many as thirty or forty. Even this liberal figure, however, would not seem to demand a list of three hundred or more as a matter of choice. In number alone such lists as those of Kraemer and of Schneider in "Pharmaceutical Plants and Their Culture" might serve to suppress, rather than arouse interest in drug growing. They not only seem discouraging in this respect but are also confusing and impractical. In "Pharmaceutical Plants and Their Culture," page 10, the author states that there is no plausible reason why such common but nevertheless very desirable drugs as taraxacum, chicory, mallow, burdock, horehound, milk weed, sambucus, stramonium, rosemary, and many others, should not be very profitably cultivated on a large scale. If cultivated upon a large scale and at the same time profitably these drugs must be held in considerable demand by pharmaceutical manufacturers. This condition certainly does not exist for more than two or three and probably not for more than one of the forms mentioned. Little difficulty has been experienced during the past years in obtaining sufficient quantities of such drugs at very moderate prices. The same is true for a large number of plants mentioned in the latter part of the same publication. This is given up to brief notes upon eight hundred and sixty-nine forms, classified as medicinal and poisonous. It is believed that for all practical purposes at least eight hundred of these forms might well be omitted.

Turning to another phase of the situation it has been found that doubtful comparisons have been made of American grown and foreign drugs. Some of these comparisons have been decidedly too liberal in certain important instances. The production of Cannabis Indica in the United States furnishes an example. The advancing price of the foreign drug has made it desirable that the United States furnish its own supply. This appeared to be one of the easiest problems in drug growing, especially in view of the statements of several authorities. These statements are to the effect that American grown cannabis is equally as active physiologically as the imported drug. These broad statements together with requests for the home grown drug have undoubtedly had a stimulating effect upon its production. Few cultural difficulties were encountered and consequently many samples of the drug soon found their way from one part of the country to another. An examination of many of these samples has failed to substantiate the statements of the above authorities. In many instances the samples were also very inferior from a physical standpoint. Samples of this drug produced by the United States Department of Agriculture in South Carolina which so far seem to represent the best American grown product, have tested 75 per cent. as active as good imported drug. Drug produced in Indiana from foreign seed does not test over 65 per cent. when compared with the drug from which the seeds were taken and some tests as low as 40 per cent. In view of this situation it does not seem fair to commercial interests to recommend at this time the raising of this drug upon a large scale. It has also been found that belladona varies greatly in percentage of alkaloids when grown in this country, and it is very probable that many sections of the country will not be suitable for its commercial production. The possibility of using the entire herb instead of the leaves in an effort to simplify its commercial production may be questionable in view of the great difference in the alkaloidal content of these parts. Samples taken from the same plot assayed as follows: leaves 0.445 per cent., leaves and stems 0.300 per cent., stems 0.065 per cent.

Information in the form of cultural directions may also be made too general. As late as May 10th a request was received for information upon the planting of one acre of digitalis. The impression had been gained that this drug could be started by open field sowing on or about this date. Such cultural directions for digitalis have been published from reliable sources upon two occasions and may or may not have been responsible for this impression. However, it should be generally recognized among investigators that digitalis is not a plant that can be propagated upon a large scale by this method. The same may be said of henbane, a drug that is probably in greater need of cultivation and improvement than any other. Immediate results would be very desirable at this time when the quality of the imported supply is extremely poor and the botanical source of the drug is questionable. Repeated failures with the commercial production of this drug have been experienced in England and still the technical difficulties of its cultivation have not been overcome. Such instructions as those of Turner, Pharmaceutical Journal, 86, 390, to the effect that henbane is not difficult to grow where the grower has a knowledge of general agriculture and Farwell, Bulletin of Pharmacy, 19, 258, that belladonna, henbane, digitalis and aconite can be grown as easily as a field of potatoes are somewhat too general for application. The cultivation of henbane in this country upon an experimental scale has resulted in such complete failure that efforts to continue its investigation have been almost abandoned. I do not agree with Schneider that from a cultural standpoint it can be associated with stramonium, cannabis, tobacco, and belladonna or that to make the culture profitable, only the flowering herb must be used. In the first place the culture of stramonium, cannabis and tobacco are extremely simple and the propagation of belladonna now fairly well developed. Much difficulty has been experienced, however, in the seeding and transplanting of henbane and in the elimination of insects from the growing plants. In the second place it is extremely doubtful whether any biennial forms can be grown profitably in this country if the second year product is demanded. In my estimation such a demand cannot be based upon strictly economic and scientific principles. The henbane situation for the past three years has demonstrated that conditions may arise which will make it not only advisable but probably necessary to pursue a vigorous search for pharmacopœial substitutes. I have demonstrated repeatedly that much of the commercial henbane is not from Hyoscyamus niger of the second year's growth and furthermore first year leaves of biennial henbane have been tested which yielded 0.089 per cent. of alkaloids. This figure is only suggestive but should be an incentive to further investigations upon the production of an annual crop of this drug. As yet however, the uncertainty of the plant under cultivation does not warrant an attempt at commercial production. The experimental stage has not been passed, and so long as this condition exists little can be said for prospective growers in the nature of direct and specific recommendations.

The foregoing conditions apply not only to henbane but in my estimation to most of the important drug plants now under investigation within the United States. The mere fact that a form is grown experimentally or for garden or decorative purposes does not signify that it can be produced successfully upon a commercial scale. The production of a few specimen plants does not involve field conditions and is no indication of the ease and rapidity with which the same form may be grown commercially. Commercial possibilities have been suggested upon these grounds and the much talked of "profit in weeds" has resulted in more talk than profit. It is now time to reduce the work on drug cultivation to an exact science, and to determine the commercial possibilities of the most promising forms in the same manner as has been done for agricultural and other economic forms. When this has been accomplished there will be ample time for recommendations to practical growers. Until then all inquiries should be met with a clear statement of the uncertainty of immediate commercial possibilities together with an idea of the exacting nature of the requirements for the various classes of medicinal plants.

*Botanical Department.*

# A STUDY OF AMERICAN GROWN BELLADONNA

By F. A. MILLER and R. N. REED

[Presented to the Pharmaceutical Division of the American Chemical Society at the meeting held in Milwaukee, March, 1913 and reprinted from the Journal of Industrial and Engineering Chemistry, Vol. 6, No. 1, January, 1914.]

A study of the experiments upon belladonna culture in the United States indicates that the quality of the drug produced based upon the percentage of total alkaloids has been fair but extremely variable. Most of these experiments have been conducted upon a small scale, though some attempts have been made at commercial production.

Rippetoe[1] reports upon a few plants grown in the Shenandoah Valley, Virginia, where belladonna was found to be fairly hardy. His assays indicate that the leaves from first-year plants grown in this locality contained 0.23 per cent. of alkaloids, the second year leaves 0.68 per cent., one average plant (leaves) 0.48 per cent. and the roots from the same plant 0.38 per cent. Schneider[2] reports upon drug grown at San Francisco, California, which assayed 0.40—0.80 per cent. for the leaves and 0.50 per cent. for the stems. He also grew some plants at Salina, California, which he claims gave a high yield. Unfortunately, we are left in doubt as to the exact nature of this "high yield" and cannot say whether he referred to relative amount of drug produced or to the alkaloidal yield. Again[3] in 1911 there is a meager report upon drug grown near Oakland, California, which is said to have been of "very high quality." Also in the 1912 report[4] from the same region the author states that he is satisfied the crop of that year will show a high percentage of alkaloids, perhaps 0.75 per cent. or more. Further, he states that some of the belladonna leaves and stems from this locality have yielded as high as 0.84 per cent. It is to be regretted that more accurate data have not been given on these experiments. Sayer[5] gives the following assays on California-grown belladonna: leaves 0.64 per cent., stems 0.29 per cent., small stems 0.43 per cent. and coarse stems 0.16 per cent., average 0.51 per cent. He quotes manufacturers as having found a variation of from 0.35 per cent. to 0.84 per cent. of alkaloids in drug from this state, with an average of 0.64 per cent. Englehardt[1] gives a series of very high figures on California drug; leaves 0.83 per cent., stems 0.85 per cent. and a mixture of leaves and stems 0.79 per cent. Evans Sons, Lescher & Webb[2] find 0.40 per cent. in leaves and 0.16 per cent. in stems from drug of commercial source. Borneman[3] describes briefly his experiments in Pennsylvania and gives assays of 0.58 per cent. for leaves, and 0.53 per cent. for roots from first-year plants.

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[1] Rippetoe, *Am. J. Pharm.*, vol. 79, p. 523, (1907).

[2] Schneider, *Proc. Am. Pharm. Assoc.* vol, 57, p. 833 (1909).

[3] Schneider. *Pacific Pharm.* vol. 5, p. 157 (1911).

[4] Schneider, *Ibid.*, vof. 6, p. 159 (1912).

[5] Sayer, *Ibid.*, vol. 4, p. 332 (1909).

In contrast with the foregoing experiments, which have been quite general as to purposes and results, is the work of Chevalier[4] on the effects of various fertilizers upon the percentage of alkaloids in belladonna and other solonaceous forms, of Ransom and Henderson[5] who have studied the influence of meteorological conditions in addition to that of commercial fertilizer and of Carr[6] who in addition to the last-named factors has performed experiments which include the light relation and its bearing upon the production of alkaloids in belladonna. In this manner the influence of environment upon the physiology of the belladonna plant is being investigated. A review of the assays recorded by various workers upon commercial belladonna for the past fifty or sixty years indicates that the alkaloidal value of this drug may range from 0.00 per cent.[7,8] to 1.32 per cent.[9] in the leaf and from 0.10 per cent.[10] to 0.86 per cent.[9] in the root. These latter figures indicate still greater possibilities in the production of alkaloids in cultivated belladonna than have yet been attained.

However, it is evident from a thorough review of the experiments upon this plant as well as medicinal plants in general, that not all means of developing them to their maximum have as yet been exhausted. It seems that the most neglected of these is the opportunity for the production of improved varieties by breeding and selection. Most of the valuable economic plants have been obtained through selection, hybridization, and adaptation. Few such forms have ever been produced through an application of the methods now being used to grow medicinal plants. It has been found that economic forms once brought to a high state of development must be intensively and continuously bred in order to retain them in this condition. Medicinal plants will doubtless be found to behave in a similar manner.

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[1] Englehardt, *Proc. Am. Pharm. Assoc.*, vol. **58**, p. 1256 (1910).

[2] Evans Sons, Lescher & Webb, *Analytical Notes*, **1906**, 7, 8.

[3] Borneman, *Am. J. Pharm.*, vol. **84**, p. 551 (1912).

[4] Chevalier, *Compt. rend*, **150**, 344 (1910).

[5] Ransom, *Proc. Eighth Int. Cong. App. Chemistry*, vol. **27**, p. 63 (1912).

[6] Carr, *Ibid.*, vol, **27**, p. 7 (1912).

[7] Francis, *Proc. Penn. Pharm. Assoc.*, **1907**, p. 66.

[8] Evans Sons, Lescher & Webb, *Analytical Notes*, **1907**, p. 8.

[9] Farr, *Pharm. Jr.*, vol. **74**, p. 398 (1905).

[10] Evans Sons, Lescher & Webb, *Analytical Notes*, **1906**. p. 7-8-9.

The behavior of active principles in selected plants and the influence upon them of various methods of breeding, together with a study of the essential factors of growth, are some of the possibilities which have led to the following experiment.

One-third of an acre was planted to belladonna in the following manner: Seeds were taken from a shipment of commercial belladonna leaves (No. 24712) and sown in the greenhouse, February 2, 1912, in seed pans. These germinated March 1st, and soon afterwards the seedlings were transplanted to flats and retained in the greenhouse until April 15th when they were transferred to cold frames. The young plants hardened off in these frames until May 20th. They were then four inches or more high and were transplanted to the open field. The soil was a stiff clay loam which had been cropped severely for a number of years. Cultivation was necessarily frequent on account of the poor physical condition of the soil. By July 12th the plants were flowering freely. As strong vigorous plants came into flower the best appearing individuals were selected and numbered. These were inbred, *i. e.* close pollinated in order to obtain seeds of known origin. Samples of leaves from these selected, inbred plants were collected for assay. They were dried at room temperature, granulated and sealed in amber bottles until assayed. Ten individual plant selections were made and assayed as follows:

| No |  | Assay percentages | No. |  | Assay percentages |
|---|---|---|---|---|---|
| 10 | B—1317 | 0.820 | 15 | B—1322 | 0.600 |
| 11 | B—1318 | 0.710 | 16 | B—1323 | 0.516 |
| 12 | B—1319 | 0.550 | 17 | B—1324 | 0.616 |
| 13 | B—1320 | 0.870 | 18 | B—1325 | 0.682 |
| 14 | B—1321 | 0.664 | 19 | B—1326 | 0.604 |
|  |  |  |  | Average, | 0.643 |

At the same time a mixed sample for assay was taken from the entire plot. This sample consisted of a mixture of leaves and stems or, speaking more correctly, of the entire herbaceous portion of the plants. This sample gave a yield of 0.30 per cent. of alkaloids. A portion of this sample was separated into leaves and stems. These parts assayed 0.39 per cent. and 0.059 per cent. respectively. Another sample taken from a restricted area of the plot and consisting of leaves only assayed 0.688 per cent. A mixed sample of the roots from this same area assayed 0.43 per cent. It has already been stated that the seeds with which the experimental plot was planted were obtained from a shipment of commercial belladonna leaf. It is interesting to note that this shipment of drug assayed 0.62 per cent.

The principal object of the experiment was to locate individual plants containing high percentages of alkaloids. It was thought that individual belladonna plants growing upon a uniform soil and under the same environmental conditions would show a marked variation in percentages of alkaloids. The variation as noted in this comparatively small number of plants is from 0.516 per cent. to 0.87 per cent. The minimum and maximum percentages have evidently not been located with these ten individuals but they furnish abundant material for further study. The inbred seeds from these plants of known yield have been planted. These resulting groups of plants, grown from pedigree seeds, will be further studied for average yields and for individual variations in the percentage of alkaloids. The behavior of these selected plants under the method described will determine the possibility of developing strains which will give a uniformly high yield of alkaloids. The problem thus resolves itself into a study of the transmission and fixation of the character of an individual plant to produce a given percentage of alkaloids, when grown under uniform and proper ecological conditions.

*Botanical Department.*

# SEASONAL VARIATIONS IN THE RESISTANCE OF GUINEA PIGS TO POISONING BY OUABAIN AND BY LIQUID PREPARATIONS OF DIGITALIS.

Reprinted from the American Journal of Pharmacy, Vol. 86, p. 7, January, 1914.

CHAS. C. HASKELL, A. B. M. D.

In a previous paper[1] it has been shown that guinea pigs are more resistant to poisoning by ouabain during certain months of the year than at others. These results were confirmed in a general way by Vanderkleed and Pittenger[2] in a subsequent publication. It is of interest to compare the results secured in this laboratory with those reported by Vanderkleed and Pittenger.

I have used male pigs exclusively, and have been compelled to employ animals differing largely in weight; the majority, however, weighed 250 grams or more, so it seems best to compare my results with those obtained by Vanderkleed and Pittenger using "large males." In order to facilitate comparison, the average minimum lethal dose has been expressed in fractions of a gram per gram body weight and put in the following tabular form:

TABLE I.

| Month | | Vanderkleeed and Pittenger. | Haskell. |
|---|---|---|---|
| Jan., | 1912. | .00000025 | .00000052+ |
| Feb., | 1912. | .00000030 | .00000037 |
| March, | 1912. | .00000032 | .00000036 |
| April, | 1912. | .00000033 | .00000040 |
| May, | 1912. | .00000033 | .00000045 |
| June | 1912. | .00000033 | .00000040 |
| July, | 1911. | .00000021 | ——— |
| Aug., | 1911. | .00000021 | .00000029 |
| Sept., | 1911. | .00000021 | .00000030 |
| Oct., | 1911. | .00000021 | .00000036 |
| Nov., | 1911. | .00000024 | .00000052 |
| Dec., | 1911. | .00000028 | .00000052 |

It is readily seen that the same general conclusion is deducible from both series of tests: the resistance of the pigs is least during the hot summer months and greatest in the cooler weather. In August, there is a difference of 38 per cent. between the lethal dose required in Indianapolis and that determined in Philadelphia, and in January, the enormous preponderance of 108 per cent. is shown by the Indianapolis lethal dose. Obviously, it is scarcely to be expected that we should secure very closely comparable results in assaying a galenical if such divergence occurs in testing a "pure principle."

In endeavoring to account for this disagreement, the technic employed should be closely scrutinized. It is a well recognized fact that testing digitalis upon frogs requires the closest attention to details and necessitates the avoidance of any disturbing factors, such as large variations in the weight of the animals and, especially, extremes of temperature. From previous statements of those who have employed the guinea pig method, one is led to infer that such extreme caution is not necessary when this method is used, and the results of Vanderkleed and Pittenger seem to show that weight and age are factors of little moment.

In all of my experiments, a solution of ouabain, 1 to 10000 in 25 per cent. alcohol was used. Vanderkleed and Pittenger do not state whether alcohol was present in the solutions they employed, but its absence would explain the smaller dose determined by them as compared to the dose I found necessary in August, because alcohol exerts a similar antagonistic action toward the absorption of subcutaneously-administered ouabain as it does toward digitalis administered in this way. Some other explanation, however, is necessary to account for the difference between the minimum lethal dose determined in Indianapolis in January (.00000052+) and that determined in Philadelphia for the same month (.00000025). The pigs used by Vanderkleed and Pittenger were kept in fairly warm quarters; while our animals were subject to considerable variations in temperature, the thermometer occasionally registering as low as 50°F. This, I believe, has an important bearing on the resistance of the guinea pigs and, together with the influence of the alcohol used in my experiments, may serve to explain the difference in the lethal dose as determined in the winter months.

Since this earlier report, the minimum lethal dose of ouabain in 25 per cent. alcohol has been determined upon guinea pigs in a number of different months and a comparison is of some interest. In table II, such a comparison is given.

TABLE II.

| Month | 1911 | 1912 | 1913 |
|---|---|---|---|
| January | | .00000052+ | .00000040 |
| February | | .00000037 | .00000042 |
| March | | .00000036 | |
| April | | .00000040 | .00000037 |
| May | | .00000045 | .00000045 |
| June | | .00000040 | |
| July | | | .00000025 |
| August | .00000029 | | .00000025 |
| September | .00000030 | .00000036 | |
| October | .00000036 | .00000042 | |
| November | .00000052 | .00000040 | |
| December | .00000052 | .00000045 | |

These figures indicate that the temperature influences the powers of resistance. During the extremely cold winter of 1911-12 the dose for November, December, and January was .00000052; while during the much milder winter of 1912-13, the lethal dose was smaller. Where comparison is possible in other months, the difference never amounts to more than 15 per cent.

Seasonal variations in the resistance of test animals may be obviated by the use of a satisfactory standard, and Vanderkleed and Pittenger suggest the use of ouabain when galenicals of the "heart tonic" group are tested upon guinea pigs. The use of ouabain is justified, however, only when it has been shown that the variation in the resistance toward poisoning by ouabain parallels that toward poisoning by the galenicals under consideration. Opportunity has occurred to determine the minimum lethal dose of a small number of samples of tincture and fluid extract of digitalis at different seasons of the year. Some of the tinctures were made by the U. S. P. method and some were made with a menstruum containing 75 per cent. alcohol. All of the fluid extracts were made with a menstruum containing 70 per cent. or 80 per cent. alcohol.

In testing the tinctures, portions were evaporated to a semi-solid consistence upon the water-bath and the residue suspended in an amount of distilled water equal to the original volume of the portion taken for evaporation. The same procedure was followed with the fluid extracts, save that the volume of distilled water was five times that of the fluid extract taken. For reasons that will be apparent later, it is desirable that the preparations be divided into two groups: one comprising preparations containing about 50 per cent. alcohol; the other comprising those containing from 70 to 80 per cent. alcohol.

The comparison of the lethal doses for two tinctures made with 50 per cent. alcohol and the lethal dose for ouabain during the same month is given in table III.

TABLE III.

| | June 1912 | Oct. 1912 | Dec. 1912 | Jan. 1913 | Aug. 1913 |
|---|---|---|---|---|---|
| Ouabain | .00000040 | .00000042 | .00000045 | .00000040 | .00000025 |
| Tincture Digitalis U. S. P. 434900 | .004 | .004 | .007+ | .0065 | .0042 |
| Tincture Digitalis U. S. P. 457579 | | | .0075 | | .0052 |

So far as can be judged by this limited number of experiments, "seasonal" variations in the resistance of guinea pigs toward poisoning by ouabain and by tinctures of digitalis made with 50 per cent. alcohol follow the same general curve. In January, 1913, the lethal dose for Tincture 434900 was .0065, while in July, 1913, it showed a decrease of 35.4 per cent.; the lethal dose of ouabain showed a decrease of 37.5 per cent. during the same time. In December, 1912, the dose of Tincture 457579 was .0075, while in July, 1913, it was 30.6 per cent. less; the dose of ouabain suffered a decrease of 44 per cent.

In testing the preparations containing relatively high percentages of alcohol, entirely different results were obtained. See Table IV, p. 177 These are so surprising that it was only after confirming them repeatedly that I could feel that they were not due to some error in testing. The tests were carried out in a manner exactly similar to dealing those with the tinctures just discussed and the difference in the behavior seems to depend upon an essential difference in the composition of preparations made with 50 and 75 per cent. alcohol respectively.

From these results, it is evident that no seasonal variation has been observed in the resistence of guinea pigs to poisoning by fluid preparations of digitalis made by percolation of the leaf with menstrua containing 70 or 80 per cent. alcohol. Tinctures made with 75 per cent. alcohol differ in several important points from those made with 50 per cent. alcohol, but it seems almost incredible that the resistance of guinea pigs to poisoning by the two should follow such different lines. A confirmation of these observations would certainly suggest that careful pharmacological study of digitalis preparations made with different menstrua would not entail a waste of time.

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TABLE IV.

| | Jan. | | Feb. | | March | | April | | May | | June | | July | | Aug. | | Sept. | | Oct. | | Nov. | | Dec. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | '12 | '13 | '12 | '13 | '12 | '13 | '12 | '13 | '12 | '13 | '12 | '13 | '12 | '13 | '12 | '13 | '12 | '13 | '12 | '13 | '12 | '13 | '12 |
| Ouabain + | 52 | 40 | 37 | 42 | 36 | | 40 | 37 | 45 | 45 | 40 | | | 25 | 29 | 25 | 36 | | 42 | | 40 | | 45 |
| Tincture Digitalis 471124 | | | | | | .0027 | | | | | | | | | | .0025 | | | | | | | |
| Tincture Digitalis 461011 | | | | | | | | | | | | | | | | 0020 | | | | | | | .0025 |
| Tincture Digitalis 467056 | | 0024 | | | | | | | | | | | | | | 0023 | | | | | | | |
| Tincture Digitalis ★ 24678 | | 0025 | | | 0025 | | .003 | | | | | | | .0026 | | | | | .0025 | | .0025 | | |
| Fluid Extract Digitalis 461015 | | | | | | | | | | | | | | .00022 | | | | | | | .00023 | | |
| Fluid Extract Digitalis 1654092 | | | | | | | | | | | | | | .00047 | | | | | | | .00032 | | |

★ Alcohol not removed from this tincture.

+ The figures following Ouabain represent fractions of a gram to the eighth decimal (.00000052).

# THE RAPIDITY OF ELIMINATION OR DESTRUCTION OF STROPHANTHUS AND DIGITALIS GLUCOSIDES BY GUINEA PIGS.

CHAS. C. HASKELL, A. B., M. D.

Reprinted from the Journal of the American Pharmaceutical Association, Vol. II, p. 1559, Dec. 1913.

In the summer of 1911, experiments were performed in our laboratory to determine whether ouabain showed any tendency to manifest the so-called "cumulative" action on guinea pigs. With this end in view, the minimum lethal dose of ouabain in 25 per cent. alcohol was determined and four pigs were given barely sub-lethal doses. These same animals were injected with varying amounts of ouabain on subsequent days, after the lapse of about 24 to 48 hours. The following protocol illustrates the results secured in all instances.

Experiment I. Black male pig. M. L. D. Ouabain 0.00000028—0.00000030 gm. per gm. weight.

9/9/11 —Wt. 260 gm.: 0.00000029 gm. ouabain at 10:57 a. m. Almost died.
9/12/11—Wt. 264 gm.: 0.00000020 gm. ouabain at 3:24 p. m.
9/13/11—Wt. 242 gm.: 0.00000020 gm. ouabain at 10:57 a. m.
9/14/11—Wt. 246 gm.: 0.00000020 gm. ouabain at 4:20 p. m.
9/15/11—Wt. 250 gm.: 0.00000020 gm. ouabain at 4:20 p. m.
9/16/11—Wt. 247 gm.: 0.00000027 gm. ouabain at 11:27 a. m.
9/18/11—Wt. 556 gm.: 0.00000028 gm. ouabain at 3:58 p. m.
9/19/11—Wt. 258 gm.: 0.00000028 gm. ouabain at 5:02 p. m.
Experiment discontinued.

It is apparent from these results that ouabain is rapidly destroyed or excreted by the guinea pig and that when twenty-four hours have elapsed a guinea pig that has previously received a large dose of ouabain is no more susceptible than is an animal that has previously received none. If, however, the second injection is made in considerable less than 24 hours after the first, the animal succumbs. This may be seen from the following experiments:

### EXPERIMENT II.

Male pig, Wt. 416 gm.
9/9/11—2:33 p. m.: 0.00000028 gm. ouabain.
5:03 p. m.: 0.00000010 gm. ouabain.
Died during night.

### EXPERIMENT III.

Male pig. Wt. 613 gm.
9/9/11—2:37 p. m.: 0.00000015 gm. ouabain.
5:07 p. m.: 0.00000026 gm. ouabain.
Died during night.

The question then arose, do the animals acquire a greater degree of resistance as a result of these sub-lethal doses? That this is not the case is shown by the following protocol:

EXPERIMENT IV.

Male pig. Wt. 439 gm.
9/11/11—10:34 a. m.: 0.00000028 gm. ouabain.
9/12/11— 3:23 p. m.: 0.00000020 gm. ouabain.
9/13/11—10:59 a. m.: 0.00000028 gm. ouabain.
4:20 p. m.: Found dead.

These results are in full accordance with the experience of Vanderkleed and Pittenger.

In June, 1912, Hatcher read his excellent and comprehensive paper on "The Persistence of Action of the Digitalins," and reported that the action of ouabain showed little tendency to persist when tested on cats, but that digitalis did show such persistence. He found that some cats, after a larger, sub-lethal dose of digitalis, showed diminished resistance for as long as a month. Experiments upon rabbits showed that these animals are not rendered more susceptible to digitalis by sub-lethal doses when several hours elapse before the second injection.

In view of these results of Hatcher's, it seemed advisable to determine the behavior of guinea pigs toward digitalis; consequently, experiments similar to those already carried out with ouabain have been performed, digitalis preparations being used for the first injection, while ouabain or digitalis was used for the second injection. The results may be tabulated as follows:

| Interval between Injections | Percent. of Average M. L. D. | | Results |
|---|---|---|---|
| | First Inj. | Second Inj. | |
| 1 day...... | 36 | 80 | Survived |
| 1 day...... | 55 | 80 | Survived |
| 2 days..... | 74 | 74 | Survived |
| 2 days..... | 92 | 37 | Survived |
| 3 days..... | 57 | 86 | Survived, almost died |
| 3 days..... | 86 | 57 | Survived, very sick |
| 1 days..... | 86 | 74 | Died (two hours) |
| 3 days..... | 84 | 89 | Died (33 minutes) |
| 3 days..... | 84 | 50 | Survived |
| 4 days..... | 75 | 75 | Died (two hours and 24 minutes) |
| 4 days..... | 45 | 75 | Died (lived more than six hours) |
| 4 days..... | 50 | 75 | Died (lived more than two hours) |
| 7 days..... | 90 | 75 | Died (lived more than two hours) |
| 7 days..... | 91 | 75 | Died (2 1/2 hours) |
| 10 days..... | 60 | 67 | Died (45 minutes) |
| 11 days..... | 90 | 89* | Died (40 minutes) |
| 11 days.... | 75 | 89* | Died (45 minutes) |
| 15 days..... | 95 | 75 | Survived |

*Three fresh pigs injected with this same dose all survived.

No guinea pig was used for a second injection until its general condition seemed normal. In a number of instances, the weight showed a decided increase between injections, but the site of the former injection was always marked by induration or an open slough.

In a recent paper, Vanderkleed and Pittenger call attention to the fact that erroneous statements have been made concerning the cost of guinea pigs for an assay of a heart tonic by Reed's method. They state that the cost should be based, not upon the total number of animals employed, but upon the number that succumbs, for it is possible to use an animal time after time until it finally succumbs.

From the results secured in our experiments, it seems that digitalis glucosides are comparatively rapidly eliminated or destroyed by guinea pigs in some instances, while in others a state of increased susceptibility is induced by large sub-lethal doses of digitalis which persists for at least eleven days and has no connection with the animal's general condition.

Therefore, the conclusion seems justified that as test animals, the use of guinea pigs which have already received an injection of digitalis is unsafe until the length of time required for complete recovery can be definitely decided. It has been my own impression (unsupported by any experimental evidence) that even after a month has elapsed, unsatisfactory results may be secured, and it has been our invariable custom to use fresh animals in the final assay of any preparation.

If uniformly satisfactory results may be obtained after a month has elapsed, it is obvious that the second use of such animals as recovered will be nearly as costly as purchasing fresh pigs, because the food and care, added to loss from natural causes, will amount to a considerable sum.

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# A BIBLIOGRAPHY OF THE DETERIORATION OF DRUGS AND PHARMACEUTICAL PRODUCTS

Presented to the Scientific Section of the American Pharmaceutical Association at Nashville, Tenn., Aug. 1913 and reprinted from the Journal of the Am. Phar. Assn., Vol. III, p. 38. January, 1914

E. G. EBERHARDT AND F. R. ELDRED

The term deterioration is here restricted to the change of therapeutic value by decomposition or loss of constituents. It is only in recent years that the matter of the deterioration of drugs and their preparations has received any considerable attention. Practically all of the systematic work on the subject is of comparatively recent date. The older results were mostly incidental to work along related lines, principally that of standardization. A study of the subject discloses the need of accurate and decisive work as there is still much of uncertainty and diversity of opinion. The importance of the subject and the great advances made in the matter of standardization justify the hope that the near future will see a marked increase of our knowledge in this field.

We do not presume to offer this as a complete bibliography of the subject, but we have tried in the time at our disposal to make it sufficiently so to give a very fair review of the field. Some of the references could be seen in abstract only but were considered definite enough to merit inclusion.

It is our purpose to continue the bibliography and to publish additional references after a sufficient interval.

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Astringent Preparations, Permanence of,—Wilbur L. Scoville,—Jr. A. Ph. A., 1912, Vol. 1, p. 334.

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Belladonna Extract, Keeping Qualities of,—Holger Thaysen,—Schweiz. Wochschr., Vol. 50, p. 605; also Apoth. Ztg., 1912, p. 528; through Chem. Absts. Vol. 7, p. 681.

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Calcium Sulphide Pills and Tablets, M. R. Schmidt & H. Engelhardt,—Proc. A. Ph. A., 1910. Vol. 58. p. 1005.

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Cannabis Indica, Notes on,—E. M. Holmes,—Phar. Jr. 1902. Vol. 68, p. 342.

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Digitalisblaetter, Wertbestimmung der,—H. Ziegenbein,—Arch. der. Pharm., 1902. Vol. 240, p. 454.
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Digitalis, Infusion of, Preservative Effect of Small Percentages of Alcohol,—C. Focke,—Pharm. Ztg., 1909. Vol. 54, p. 757; through Proc. A. Ph. A. Vol. 58, p. 90.
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Digitalis, Some Points as to Time of Gathering of the Leaves and the Keeping Properties and Standardization of the Tinctures, etc,—Gordon Sharp & J. Lancaster,—Phar. Jr., 1911, Vol. 86, p. 102.

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Digitalis, The Physiological Standardization of, from the Point of View of the Pharmacist,—Robt. R. Hallaway,—Phar. Jr., 1909, Vol. 83, p. 801.

Concludes that six months is a safe time limit for the tincture.

Digitalis, The Variability of,—Hale,—Proc. Am. Ph. Assn., 1910, Vol. 58, p. 925.

Excessive drying of drug not required. Preparations seem to deteriorate little if made with 70% alcohol.

Digitalis, Tr., Its Potency and Keeping Properties,—Alexander Goodall,—Br. Med. Jr., 1912, Vol. 1, p. 887; through Am. Jr. Med. Sci., Vol. 144, p. 299, 1912; also Chem. Absts., Vol. 7, p. 536.

Tinctures retain activity for about one year, after 18 months some are under standard, and after 22 months all of them.

Digitalis, Tincture of, Necessity of Protection from Light,—C. Focke,—Phar. Ztg., 1904, Vol. 49, p. 543; through Proc. A. Ph. A., Vol. 53, p. 599.

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Digitalis Tincture,—Moran,—Med. Chronicle, 1911-12, Vol. 55, p. 1.

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Drugs, Some Experiences in the Testing of, by Bio-Chemical Methods, with Special Reference to Digitalis, Squill and Strophanthus,—Wm. Martin,—Pharm. Jr., 1909, Vol. 83, p. 149.

Digitalis drug if properly prepared and stored, retains activity for many years and the tincture for nine or twelve months. Tincture Squill begins to deteriorate sooner than digitalis, but the change is slower. Tinct. Strophanthus remains unimpaired for many years.

Drugs, The Bio-Chemical Standardization of,—W. E. Dixon and G. S. Haynes, —Proc. Therap. Soc., 1905; through Pharm. J., Vol. 75, p. 754.

The variability of commercial tinctures may be due to deterioration. Standard tinctures were unchanged after eight months.

Drugs, The Deterioration of,—Jr. Am. Med. Assn., 1912, Vol. 59, p. 959.

Communication from the Council on Pharmacy and Chemistry of the A. M. A., recommending the dating of certain preparations.

Drugs, the Preservation of,—Linwood A. Brown, Bull. No. 150. Ky. Agr. Exp. Sta. of the State Univ.

Discusses the storage of crude drugs and various pharmaceutical preparations.

Ergot.—R. Kobert,—Central. f. Gynækol., 1886, Vol. X, p. 306.

Concludes that ergot more than a year old has no action on the uterus. (Seen in reference only.)

Ergot,—Kehrer,—Arch. f. Exp. Path. u. Pharm., 1908, Vol. 58, p. 366.
Within one year the activity sinks to 1 7 of the original strength and in two years to 1 15. (Seen in reference only.)

Ergot, An Experimental Study of the Pharmacology of,—Wood & Hofer,—Arch. Int. Med., 1910, Vol. 6, p. 388.
A fluid extract exposed to air deteriorates extremely rapidly. Under the most favorable conditions the loss of strength approximates 10% a month.

Ergot; A Symposium,—Jr. Am. Phar. Assn., 1912, Vol. 1, p. 653.
A discussion of various phases of the subject at the meeting of the N. Y. Branch of the Am. Phar. Assn., May 13, 1912, by H. H. Rusby, C. E. Vanderkleed and Cornelius De Jonge.

Ergot, Enzymes in,—J. Schindelmeyer,—Apoth. Ztg., 1909, Vol. 24, p. 837; through Chem. Absts., Vol. 4, p. 1083; also Proc. A. Ph. A., Vol. 58, p. 157.
As complete and rapid drying prevents deterioration, this may be caused by enzymes, the presence of two being demonstrated.

Ergot, Experiments on Deterioration of,—Meulenhoff,—Nederl. Tijdschr. v. Pharm. (abst.) Phar. Rundsch., 1900, Vol. XXVI, pp. 738-772.
Believes that ergot under suitable conditions retains considerable activity for five years. (Seen in reference only.)

Ergot, How long does Liquid Extract of, Retain its Pharmacological Activity,—Phar. Jr., 1908, Vol. 80, p. 82.
By clinical trial no deterioration was apparent after one year. It is not necessary that ergot preparations be kept longer than twelve months.

Ergot, Intravenous Injection of,—Sollman and Brown,—Jr. Am. Med. Assn., 1905, Vol. 45, p. 229.
Comparisons were made of eleven different preparations of ergot, both fresh and old. Age of drug seems to make no difference.

Ergot, Its Production and Collection in Russia, D. A. Ruffman and T. Maben, —Pharm. Jr., 1908, Vol. 80, p. 247.
Effect of age on ergot is discussed.

Ergot preparations, A New and Reliable Method for the Preservation of,—Paul S. Pittinger and Chas. E. Vanderkleed,—Jr. Am. Pharm. Assn., 1912, Vol. 1, p. 799.
Contact with air is the most potent cause of deterioration. A vacuum method of storage will preserve for a considerable time.

Ergot, Preservation of.—Dragendorff,—(abst.) Proc. Am. Phar. Assn., 1877, Vol. 25, p. 119.
The oxidation of the fat causes deterioration; would retain activity if fat were removed.

Ergot, Fluidext., Relative Strength of Fresh and Old Samples of,—C. C. Haskell and C. R. Eckler,—Jr. Am. Phar. Assn., 1912, Vol. 1, p. 412.
Properly kept, fluid extract retains activity for two to two and one-half years, after which deterioration becomes apparent, amounting to 50% in four or five years.

Ergot, Stability of Pressed,—John Moss,—Yearbook of Pharm., 1885, p. 410: through Proc. Am. Phar. Assn., 1886, Vol. 34, p. 371.
A sample of ergot, pressed to remove oil, retained its potency for six and one-half years.

Ergot, Stability of, when Deprived of Fixed Oil,—Hermann Werner,—Phar. Ztg., 1881, p. 397; through Proc. Am. Phar. Assn., 1882, Vol. 30, p. 142.
Ergot so treated kept its virtues unimpaired for two years or more.

Ergot, Standardization of,—H. C. Wood and C. A. Hofer,—Univ. of Pa. Med. Bull., 1909, Vol. 21, p. 347.

Of twelve fluid extracts examined, two were active. Both drug and fluid extract deteriorate rapidly.

Ergot, The Chemical Assay of Fluidextracts of,—J. R. Rippetoe,—Am. Jr. Phar., 1910, Vol. 2, p. 119.

There is a marked difference between fresh and old preparations.

Ergot and Its Fluidextracts, The Keeping Qualities of;—H. C. Wood, Jr.,—Am. Jr. Phar., 1911, Vol. 83, p. 172.

Fluidextract deteriorates rapidly, from 1.3% to 3.5% per week, according to conditions. Should not be kept longer than one year.

Ergot, The Physiological Standardization of,—C. W. Edmunds and Worth Hale,—Bull. No. 76, Hygienic Lab., U. S. Pub. Health and Mar. Hosp. Service, 1911.

Fluidextracts vary greatly. Preparations should be marked with date of manufacture.

Ergot, The Rate of Deterioration of Fluidext. of,—Wood.,—Proc. Am. Ph. Assn., 1910, Vol. 58, p. 883.

Fluidextract under the most favorable conditions loses 45% to 50% in the first year. Under unfavorable conditions the same loss occurs in three months.

Extracts, Belladonna and Henbane, Variation of Alkaloid on Keeping,—G. Ortlieb,—Phar. Ztg. 1903, p. 162; through Proc. Am. Phar. Assn., Vol. 51, p. 621.

Alkaloidal value diminishes on keeping.

Extracts, Variation of Alkaloids in Some,—Jean Fricotel,—Bull. Sci. Pharmacolog., 1908, Vol. 15, p. 687; through Chem. Absts., Vol. 4, p. 234; also Proc. A. Ph. A., Vol.57, p. 73.

Moist extracts show decrease in alkaloid after eight months in conium, belladonna, stramonium, opium and aconite.

Fluidextracts, Value of Glycerin as Solvent and Preservative,—Richard Firlas, —Apoth. Ztg., 1909, Vol. 24, p. 721; through Proc. Am. Phar. Assn., Vol. 58, p. 85.

Alkaloid diminishes more rapidly in fluidextracts made without glycerin.

Folia Belladonna, F. Hyoscyami u. F. Stramonii, Ueber die Aufbewahrungsdauer von,—R. Gaze,—Apoth. Ztg., 1912, p. 402; through Phar. Zentralh., Vol, 53, p. 1406.

The powdered drugs kept in glass-stoppered bottles at room temperature and assayed at intervals during two years showed no loss.

Galenical Preparations Containing Alkaloids, Stability of,—Dohme & Engelhardt,—Proc. Am. Phar. Assn., 1910, Vol. 58, p. 782.

All fluidextracts tested kept their alkaloidal strength well, excepting those of coca and physostigma.

Galenical Preparations of the U. S. P., Detailed Investigations of Certain, with Special Reference to their Stability,—Dr. M. Clayton Thrush,—Pharm. Era, 1912, Vol. XLV, p. 750.

A general discussion of results obtained by others with conclusions by the author.

Galenical Preparations, The Stability of,—Dohme,—Am. Drug., 1909, Vol. 55, p. 37.

A number of products of various ages were assayed. No appreciable deterioration found except in powd. ext. physostigma, fluidextracts of coca and aconite and tincture aconite.

Galenicals, Deterioration of,—Phar. Era, 1912, Vol. 45, p. 741. Editorial.

Galenicals, The Potency and Keeping Properties of Some, as Determined by Physiological Tests,—Alexander Goodall,—Phar. Jr., 1912, Vol. 89, p. 130.
Tincture digitalis not reliable after one year. Tr. strophanthus retains activity at least three years. Tr. Squill may deteriorate after two years.

Heart Tonics, The Pharmacological Assay of,—Houghton & Hamilton,—Proc. Am. Ph. Assn., 1909, Vol. 57, p. 773; also Am. Jr. Phar., Vol. 81, p. 461.
An investigation of the potency of preparations of digitalis, squill, convallaria and strophanthus, showing loss with age in digitalis.

Hydrastis, Ausscheidungen in Extractum Fluidum,—C. Linde,—Arch. d. Phar., 1898, Vol. 236, p. 698.
The precipitate deposited in fluidextract consists principally of berberin and hydrastin.

Hydrastis, Extractum, Fluidum.—Kunze,—Apoth. Ztg., Vol. 28, p. 223; through Chem. Absts., 1913, Vol. 7, p. 2089.
A specially prepared sample changed from 2.86% hydrastine to 2.19% in one year.

Hydrastis, Fluidextract of,—L. Derlin,—Apoth. Ztg., Vol. 25, p. 190; also G. Fromme, Ibid. Vol. 25, pp. 250, 274, 303; through Chem. Absts., Vol. 4, p. 1894.
The alkaloidal content of five samples out of six did not change more than .1% within six to twelve months.

Hydrogen Dioxide at Present on the Market, The Quality of Medicinal,—Kebler,—Proc. Am. Ph. Assn., 1910, Vol. 58, p. 903.
Investigation extending over one year showed progressive loss of strength.

Hydrogen Dioxide Solutions, Examination of,—L. F. Kebler, L. E. Warren and E. A. Ruddiman,—Bull. 150, Bu. Chem., U. S. Dept. Agriculture.

Hydrogen Peroxide,—C. B. Jordan,—Proc. Ind. Phar. Assn., 1912; also Jr. Am. Phar. Assn., Vol. 2, p. 344.
Shows result of keeping under various conditions.

Hydrogen, Peroxide of,—A. R. L. Dohme and Engelhardt,—Am. Jr. Phar., 1910, Vol. 82, p. 69.
Discusses various means of preservation.

Hydrogen, Peroxide,—Phar. Era, 1913, Vol. 46, p. 1.
Editorial discussion giving a number of conclusions.

Hydrogen Peroxide, Preservation by Acetanilide and Time Sale Limit,—Ph. Era, 1913, Vol. 46, p. 12.
A symposium of replies to queries sent out by the Era.

Hydrogen Peroxide, Production, Past and Present,—J. S. Brewer,—Jr. Am. Phar. Assn., 1912, Vol. 1, p. 1002.
A general discussion of the subject.

Hydrogen Peroxide, Solution of, containing Acetanilide,—C. H. LaWall,—Am. Jr. Phar:, 1905, Vol. 78, p. 582.
Calls attention to decomposition of acetanilide when used as preservative.

Hydrogen Peroxide Solution, Permanence and Acidity of,—L. W. Andrews,—Trans. Am. Inst., Chem. Eng., Vol. 2, p. 238; through Chem. Absts., 1911, Vol. 5, p. 759.
Samples of poor keeping qualities lose 10% to 50%.

Hydrogen Peroxide Sol., Reliability of the Commercial Sorts,—Robert C.

Purcel, Proc. Pa. Phar. Assn., 1912, p. 143; through Proc. Am. Phar. Assn., Vol. 51, p. 612.

Shows deterioration in 6 months on four samples.

Hydrogen Peroxide, The Preservation of,—J. H. Walton and R. C. Judd,—Orig. Com. 8th Int. Cong. Appl. Chem. (Appendix), Vol. 26, p. 621 (Abst.)

A study of the influence of various factors on decomposition.

Indian Hemp, Experiments on the Cause of the Loss of Activity of,—C. R. Marshall,—Phar. Jr., 1909, Vol. 82, p. 418.

Oxidation is the cause of loss of activity. Cannabis and preparations should be stored in hermetically sealed containers.

Ipecac, A Study of, and Review of its Literature, R. R. D. Cline,—Southern Phar. Jr., Vol. 4, pp. 11 and 56.

If kept in a cool dry place the drug retains its activity. The pharmaceutical preparations often deteriorate enormously.

Ipecac, Ext. Liquidum, B. P.,—J. W. Thompson, Phar. Jr., 1900, Vol. 64, p. 54.

Finds liquid extract unchanged after 6 or 7 months.

Ipecacuanha, Stability of the B. P. Liquid Extract,—H. Wippell Gadd,—Chem. and Drug., 1901, p. 21; through Proc. Am. Phar. Assn., Vol. 49, p. 574.

The assay remains unchanged after four months in liquid extract, wine and vinegar of ipecac.

Ipecacuanha, The B. P. Preparations of,—R. Glode Guyer,—Phar. Jr. 1899, Vol. 63, p. 622.

Deterioration noted in liquid extract and other preparations.

Jodtinktur, Ueber die Haltbarkeit der,—L. Johannessen,—Pharm. Zentralh., 1913, Vol. 54, p. 221.

Determinations of free I and acidity (HI) at intervals of one week in different tinctures. KI increases stability.

Medicinal Plants, Retrogression of Active Substance in, by the Action of Enzymes, -P. Lami,—Bull. Chim. Farm., 1911, Vol. 50, p. 835; through Chem. Absts., Vol. 6, p. 1809.

Suggests prevention of change by sterilization with hot ethyl and methyl alcohol vapors and subsequent drying.

Medicinal Plants, Sterilization and Drying of,—E. Bourquelot,—Jr. Pharm. Chim., Series 7, Vol. 3, p. 149; through Chem. Absts., 1911, Vol. 5, p. 2412.

Loss of active substance by enzyme action amounted to 31% in aconite leaves, 21.7% in digitalis, 10% in aconite root, 26.4% in colchicum bulbs.

Medicinal Plants, Sterilization of,—Abst. of a lecture delivered before the Academy of Medicine, Paris, by Prof. E. Bourquelot,—Phar. Era, 1912, Vol. 45, p. 599.

Alkaloid is lost through the action of ferments.

Mutterkornwirkung, Beitræge zur Kenntniss der,—A. Grunfeld,—Arb. d. Pharmakol. Inst., Dorpat, 1892, Vol. 8, p. 108. (Seen only in reference.)

Concludes that under ordinary conditions ergot is worthless after 6 months.

Mutterkorns, Geburtsklin. Untersuch. u. d. Haltbarkeit. des,—A Bishofsberger,—Diss. Bern., 1897. (Seen only in reference.)

The effect of one and two year old drugs was compared clinically, showing a moderate decrease of activity in the older drugs.

Narcotic Extracts, Study of Some,—E. Carlinfanti,—Bull. Chim. Farm., Vol. 51, p. 777; through Chem. Absts., 1913, Vol. 7, p. 1400.

Assays of extracts of aconite, belladonna, hyoscyamus and nux vomica show practically no change in two years.

Nitroglycerin in Tablets, Comments on Some Official Standards and Tests, – Henry L. Bernegau,—Am. Jr. Ph., 1907, Vol. 79, p. 555.

A number of lots of tablets showed no loss in nine months.

Nitroglycerin Tablets, Digitalin Tablets, and Fluidextract Ergot, Physiologic Assay of,—C. H. Edmunds and Geo. B. Roth,—Jr. Am. Med. Assn., 1908, Vol. 51, p. 2130.

Nitroglycerin tablets lost none of their strength in two years. Fl. Ext. Ergot showed variation in strength. One sample apparenty had deteriorated when tested after three months.

Opium, The Keeping Qualities of Powdered,—L. Debordeaux,—Jr. Phar. Chim., Series 7, Vol. 6, p. 491; through Chem. Absts., 1913, Vol. 7, p. 680.

Shows increase in insoluble morphine with age; also a decrease in total morphine apparently due to oxidation.

Pepsin and Pepsin Preparations, Systematic Observations on,—C. T. Nixon, —Proc. Am. Phar. Assn., 1910, Vol. 58, p. 1264.

Alcoholic preparations lost 10% of activity in three weeks, 20% in five weeks, and were practically inert after one year.

Pepsin, Conservation of the Activity of, in Elixirs of Pepsin,—E. Thibault,—Jr. Pharm. Chim., Series 7, Vol. 1, p. 480; through Chem. Absts., 1911, Vol. 5, p. 1823.

Claims that pepsin is weakened by prolonged contact with alcohol stronger than 10%.

Pepsin, Effect of Alcohol in Solution of,—Eugene Thibault,—Jr. Pharm. Chim., Series 6, Vol. 15, p. 161; through Proc. Am. Phar. Assn., 1902, Vol. 50, p. 1081.

Shows marked diminution of peptic activity after prolonged contact with weak alcohol.

Pepsin, Lactated, Elixir of,—W. A. Pearson,—Proc. Am. Phar. Assn., 1909, Vol. 57, p. 905.

Tests made as to amylolytic and proteolytic activity. A preparation containing 15% alcohol shows only about one-half the theoretical pepsin strength after a few weeks.

Pepsin, Pancreatin and Combinations of these Ferments, Laboratory Studies of,—A. Zimmerman,—Jr. Ind. and Eng. Chem., 1911, Vol. 3, p. 750.

Pepsin and pancreatin with the proper degree of acidity can be kept in the same solution for at least two and one-half years, without injury to either.

Pepsin Solutions, Effect of Hydrochloric Acid on,—Liebmann & Johannssen, —Ugeskrift for Lager, 1911, No. 25; through Phar. Zentralh., Vol. 53, p. 263; also Phar. Era, 1912, Vol. 45, p. 313.

The presence of HCl causes loss of peptic activity proportionate to the acidity and the time of standing.

Pepsin, Stability of Peptonizing Power of Liquid Preparations of,—A. Petit and A. L. Petit,—Jr. Phar, Chim., Series 7, Vol. 1, p. 150; through Proc. A. Ph. A., Vol. 58, p. 393; also Chem. Absts., Vol. 4, p.2977.

Experiments extending over more than six years show that elixirs and wines retain digestive power for years practically undiminished.

Pharmaceutical Preparations, Causes of Deterioration to be Avoided,—Leon C. Fink,—Bull. Phar., 1898, p. 105; through Proc. Am. Phar. Assn., Vol. 46, p. 652.

Deterioration may be avoided by proper care to avoid the influence of air. moisture, cold, heat and sunlight.

Pharmaceutical Preparations, Influence of Enzymes in the Production of,—M. Winckel,—Schweiz. Wochschr., Vol. 47, p. 705; through Chem. Absts., 1910, Vol. 4, p. 1085.

On the influence of the elimination of enzyme action in conserving digitalis and ergot.

Pharmaceutical Preparations, Note on the Loss of Strength of Some, by Long Storage,—R. A. Cripp,—Ph. Jr., 1907, Vol. 78, p. 519.

Shows the results of tests at intervals on Acet. Scillae, Liq. Ammon. Fort., Liq. Ammon. and Tr. Quin. Ammon.

Phosphorized Oil, The Permanence of,—Hugo Korte,—Phar. Ztg., Vol. 53, p. 655; through Chem. Absts., 1909, Vol, 3, p. 98.

Under some conditions the phosphorous content diminished rapidly. Light seems to exert more influence than air.

Report of Analysis of Preparations under the Food and Drug Law,—L. E. Sayre,—Trans. Kansas Acad. Sci., Vol. 22, p. 100.

To answer the question as to what constitutes deterioration, Prof. Sayre proposes to define it as a deviation from the professed standard * * * as may be determined by microscopical, chemical and macroscopical examination.

Sanguinaria, Assay of Fluidextract of,—H. B. Meade,—Jr. Am. Phar. Assn., 1912, Vol. 1, p. 134.

The alkaloid content fell from 2.58 gm. per 100 cc. on Feb. 21 to 2.23 gm. June 1.

Sarsaparillen, Ueber die Pharmakologische Bedeutung und die Biologische Wertbestimmung der, und ihnen verwandter Drogen.—Ber. d. Deut. Phar. Gesell, 1912, Vol. 22, p. 205,—R. Kobert, –Quillaja, p. 215; Senega, p. 218; Sarsaparilla, p. 227.

Testing the haemolytic effect on various kinds of blood, the author finds thirty year old quillaja bark practically unchanged. Thirty year old senega is only 1 5 the strength of fresh root, and Honduras and Vera Cruz sarsaparillas of the same age about 1/5 to 2/3 respectively.

Sirupus Ferri Iodati.,—P. Bohrisch,—Pharm. Zentralh., 1913, Vol. 54, pp. 343, 371.

A general discussion as to preparation and preservation, with assay on 19 samples, fresh, at six months and twelve months.

Solanaceous Extracts, Keeping Properties of,—Ribaut,—Bull. d. Sci. Pharm., 1908, Vol. 15, p. 495; through Phar. Jr., 1908, Vol. 81, p. 588; also Proc. Am. Phar. Assn., Vol. 57, p. 74.

Loss observed after four years: Ext. Belladonna leaves, 3% to 45%; Ext. Hyoscyamus, 69%; Ext. Stramon. leaves, 8% and 31%. Ext. Bellad. root, 1% to 12%; Ext. Hyoscyamus seed, 25%.

Spirit of Nitrous Ether, Inquiry into Cause of Change,—E. H. Farr and R. Wright,—Trans. Br. Phar. Conf., 1901, p. 447; through Proc. Am. Phar. Assn., Vol. 50, p. 749.

Deterioration is extremely rapid under ordinary conditions of storage.

Spirit of Nitrous Ether B. P., Progressive Deterioration in Containers,—S. F. Burford,—Chem. and Drug., 1908, p. 108; through Proc. Am. Phar. Assn., Vol. 50, p. 749.

Loss observed at intervals of 30 days during 6 months, when it had lost 82%.

Spirit of Nitrous Ether, The Keeping of, and a Suggestion for a Change in Formula,—Linwood A. Brown,—Am. Drug., 1911, Vol. 59, p. 215.
Gives results of assays made at intervals of two weeks on twelve different samples stored under varying conditions.

Spirit of Nitrous Ether, Deterioration of,—F. L. Shannon,—Jr. Am. Phar. Assn., 1910, Vol. II, p. 83.
Results are given on seven samples kept under average conditions and assayed at intervals of three months during fifteen months.

Spirit of Nitrous Ether, Preservation of,—G. E. Show,—Phar. Jr., 1903, Vol. 71, p. 236.
Properly stored, there is but slight loss. Under unfavorable conditions the loss in 14 days is from 12 1/2% to 90%.

Standard Pharmaceuticals, Deterioration of Some, H. E. Barnard,—Ph. Rev., 1908, Vol. 26, pp. 308, 321.
Experiments show an increase in strength with age in spirit of camphor and tincture of iodine and decrease in strength in lime water and ammonia water.

Strophanthus, Tr.,—Robert A. Hatcher,—Jr. Am. Med. Assn., 1907, Vol. 48, p. 1177.
No deterioration found in tincture even after sixteen years. The seeds do not undergo deterioration in keeping for several years.

Strophanthus, Tincture of,—A. R. Cushny,—Br. Med. Jr., 1912, Vol. II, p. 685.
Tinctures of digitalis, squill and particularly strophanthus, deteriorate rapidly after dilution with water.

Sydenham's Laudanum, The Preparation and Preservation of,—M. Debourdeaux,—Jr. Pharm. Chim., Series 7, Vol. 6, p. 544; through Chem. Absts., 1913, Vol. 7, p. 2093.
After storing, showed a loss of 6% of its morphine which was not found in the precipitate. After one year there was an additional loss of 10%. A 2 1/2 year old tincture had lost 17% and one 20 years old 44%.

Tinctures, Do they Deteriorate with Age?—Drug. Cir., 1913, Vol. 57, p. 389; reprinted from the Chem. Drug.
A discussion of various preparations as to stability.

Tincture Iodine,—H. Wastenson,—Svensk. Farm. Tidskrift., Vol. 17, pp. 68, 81, 113; through Chem. Absts., 1913, Vol. 7, p. 1952.
Increase of HI determined in a number of tinctures at intervals during eight weeks: KI retards deterioration.

Tincture of Iodine,—E. H. Gane,—Am. Drug., 1904, Vol. 44, p. 39.
Impurities in alcohol cause deterioration. With good alcohol the loss is about 1% of I in one year.

Tincture of Iodine,—C. H. LaWall,—Proc. Am. Phar. Assn., 1907, Vol. 55, p. 156.
The U. S. P. VIII tincture is a stable preparation under practically all conditions.

Tincture of Iodine,—L. F. Kebler,—Jr. Am. Phar. Assn., 1913, Vol. II, p. 514; Jr. Ind. Eng. Chem., Vol. 5, p. 484.
Attention is called to the preservative effect of KI and the variation in samples examined. The limit of permissible variation is discussed.

Tincture of Iodine, Alteration on Standing,—C. Courtot,—Jr. Pharm. Chim., Series 7, Vol. 1, pp. 297, 354; through Chem. Absts., 1911, Vol. 5, p. 564.
Three experimental tinctures were examined monthly for one year. The iodine decreased from 67.5 gm. per liter to 54.6, 54.6 and 58.4 gm. respectively.

Tincture of Iodine, Changes in, on Storage, etc.,—Th. Budde,—Apoth. Ztg., Vol. 27, p. 203; through Chem. Absts., 1912, Vol. 6, p. 1493; also Pharm. Ztg., 1912, Vol. 57, p. 176; through Jr. Am. Phar. Assn., Vol. 1, p. 881.
Loss amounts to 20% in 9 months retarded by addition of KI or NaI, but noticeable nevertheless after six months.

Tincture of Iodine, D. A. B.—V. Weiblitz;—Phar. Ztg., Vol. 57, p. 734; through Chem. Absts., 1913, Vol. 7, p. 535.
Free iodine content with age increases materially. Limits of variation are needed.

Tincture of Iodine, Influence of Light and Air,—C. Hugenholtz, —Phar. Ztg., 1907, Vol. 52, p. 222; through Proc. Am. Phar. Assn., Vol. 55, p. 697.
Deterioration is greater if kept in full bottles protected from light.

Tincture of Iodine—Influence of Temperature and Light, etc.—C. Courtot,—Jr. Pharm. Chim., Series 7, Vol. 2, p. 344; through Chem. Absts., 1911, Vol. 5, p. 2525.
Changes shown in tinctures of the French Codex 1908. Light has no influence, but temperature effects change.

Tincture of Iodine, Observations on Commercial,—Agnes Dunning and L. E. Sayre,—Am. Drug., 1909, Vol. 55, p. 211.
An investigation of the effect on different kinds of stoppers. With cork stoppers there is progressive concentration.

Tincture of Iodine Therapeutically Considered,—C. Courtot,—Jr. Pharm. Chim., Series 7, Vol. 1, p. 439; through Chem. Absts., 1911, Vol. 5, p. 1492.
There is concentration with age by evaporation.

Tincture of Iodine, The Stability of Free Iodine in,—Droste,—Pharm. Ztg., Vol. 57, p. 166; through Chem. Absts., 1913, Vol. 7, p. 215.
The alkalinity of glass container is responsible for loss.

Tincture of Iodine, U. S. P. VIII,—Theo. D. Wetterstroem, —Proc. Ohio State Pharm. Assn., 1908, p. 52; through Proc. Am. Phar. Assn., Vol. 57, p, 115.
KI retards change. Strength increases by evaporation.

Tinkturen, Ueber die Zweckmæssigkeit von Perkolation oder Maceration zur Herstellung von,—J. Herzog,—Ber. d. Deutsch. Phar. Ges., 1906, Vol, 16, p. 359.
Discusses the influence of air, light and temperature on tinctures.

Tinctures and "Alcoolatures," A Comparative Study of the Active Principles in Some,—E. Dejean,—Jr. Pharm. Chim., Series 6, Vol. 29, p. 274, through Chem. Absts, 1909, Vol. 3, p. 1909.
Attention is called to loss of alkaloid in drugs during drying and the resulting lack of activity in tinctures made from them as compared with "mother tinctures" from green plants.

Wild Cherry Bark, Valuation of,—A. B. Stevens,—Proc. Am. Phar. Assn., 1896, Vol. 44, p. 215.
Differences in assay point to deterioration.

Wild Cherry Bark,—A. B. Stevens,—Proc. Am. Pharm. Assn., Vol. 47, p. 184,
Deterioration shown by assays at intervals of one year.

Wild Cherry, Deterioration of Syrup of,—J. Graham French,—Proc. Penn. Pharm. Assn., 1912; through Am. Jr. Pharm., 1913, Vol. 85, p. 82.
Hydrocyanic acid disappears within 3 or 4 months.

*Scientific Division.*

# THE STRUCTURE AND DIAGNOSTIC VALUE OF THE STARCH GRAIN

R. B. HARVEY

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In view of their common occurrence in plant tissues, starch grains have been used, especially in Pharmacognosy, to differentiate between plants. While there is a great variation in the size, shape and structure of starch grains, those of different members of a genus, or even of a family, often show a similarity. Hence, these group characteristics often may be used to identify a given starch as belonging to a certain group of plants. As medicinal action or value varies greatly between closely related members of the same genus, it is of the highest importance to establish the authenticity of the species, and in this determination the starch grain is often of the greatest diagnostic value.

The characters most often used in the identification of starches are the size, shape, and markings of the grains. The most distinct markings are the hilum and the concentric layers of starch. It is very commonly stated that the hilum is the point of attachment of the grain, and that it occupies a position on the surface, while in reality it is the part first formed, and is marked by a fissure or cleft in the interior, caused by the loss of moisture, and shrinking of the central portion.

The starch grain has a structure somewhat similar to that of the sphaero-crystal, and like it grows by the apposition of new materials. According to the best authorities, the grain is made up of minute crystals or miscellæ of soluble starch, or granulose, imbedded in a frame work of starch cellulose. Alternate layers seen in many grains, contain a greater proportion of granulose, and hence stain more deeply with iodine solution. This structure of the grain was demonstrated by the action of such solvents as chloral hydrate or diastatic solutions which dissolve the granulose very rapidly, and leave a frame work of starch cellulose, of the same size as the original grain, but lacking the substances which produce the characteristic color with iodine solution.

Hence, it was stated by Meyer that the soluble starch was distributed throughout the grain in very small crystals or trichites. A consideration of the behavior of the grain upon swelling certainly demands a structure of this nature. The methods used in the preparation of this paper, and the results obtained agree with this structure, but indicate that the crystals or trichites of soluble starch may be of a considerable size. Since the crystals have nearly the same refractive index as the remainder of the grain, they cannot be detected without special treatment.

The structure of the starch grain is most easily seen in such grains as those of the common potato, or of arrowroot, which show the layers of starch most plainly, or those of Bamboo Brier root, which show the characteristics of the central portion. When these starches are stained with certain dyes, of which safranin is the best example, and the excess of stain is extracted, the central portion retains the stain, and the crystalline nature of this portion becomes evident. In the center of the grain, crystals are seen arranged with their longer axes extending radially from the hilum. The clefts in this crystalline mass mark the hilum, and the fissures caused by the loss of moisture follow the longer axes of the crystals, forming the fan-shaped markings often seen in the unstained grain. Fig 1. When the grains are kept moist at 50° C. for some time, the moisture passes through the outer layers, causing but little change in their structure, but when the crystalline portion is reached, this swells up and slowly dissolves from its surface, becoming surrounded by a zone which stains deeply with iodine solution, indicating the presence of soluble starch. The solution and swelling of this inner portion pushes upward the outer layers, which finally rupture at the thinnest point, that is, at the hilum end in eccentric grains. It therefore appears that the greater moisture content of this portion is due to its higher solubility, and indeed this part may be entirely dissolved and carried away in solution before the outer layers are affected. This is commonly the case in such grains as those of the common yam, or of Bamboo Brier root. Fig 4.

Something of the chemical nature of the various layers of the grain can be determined by treating with the following easily reducible silver solution, which may be compared to Fehling's alkaline cupric tartrate solution:

| | |
|---|---|
| Silver Nitrate | 1 gm. |
| Water | 15 Cc. |
| Ammonia Water q. s. | |

Add the ammonia water to the solution of the silver nitrate until the precipitate first formed just dissolves.

| | |
|---|---|
| Potassium and Sodium Tartrate | 2 gm. |
| Water | 15 Cc. |
| Dissolve | |

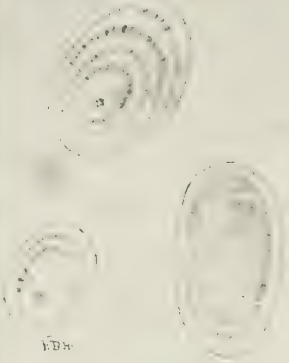


Fig. 2

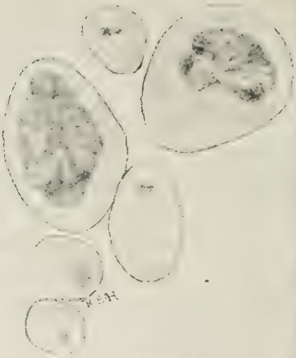


Fig. 1

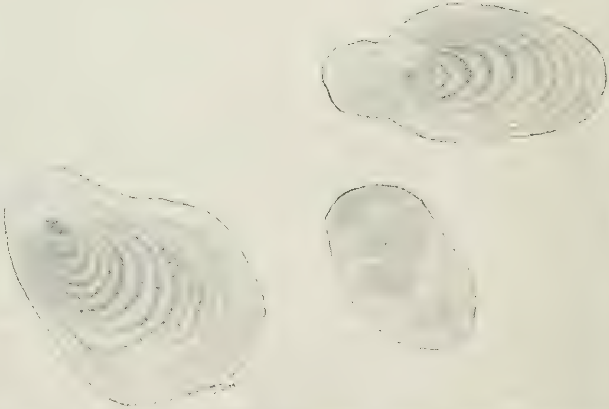
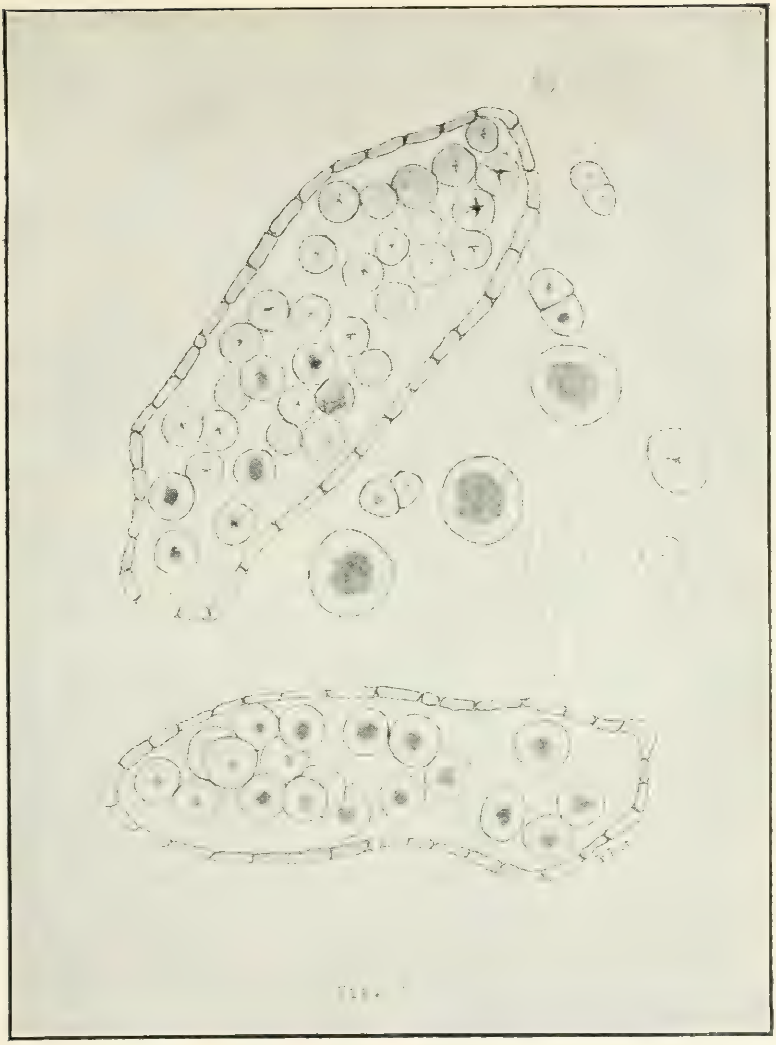


Fig. 3

When starch grains are treated for a time with a small amount of the ammoniacal silver solution to allow the silver salt to be evenly distributed throughout the grain, and then an equal amount of the artrate solution added, the silver salt is reduced by the granulose present in the grain, giving stains varing in color from purple through shades of brown to black, depending upon the state of reduction. Reduction occurs in a few hours at ordinary temperature, or may be quickly accomplished by warming very slightly although too strongly heating causes complete reduction of the solution and total blackening of the grain.



If it is desired to make permanent mounts by the above method, the starch should be stained in bulk until the proper depth of color is reached, and then the soluble and unreduced silver salt washed out with water to prevent further reduction. The grains may then be dehydrated and mounted permanently in balsam.

When starch grains are treated according to the above method, alternate layers and the central portion reduce the silver solution most rapidly, showing the presence of reducing substances which cause a deposit of silver. Fig 2. The alternate layers which stain most deeply with iodine solution take on a granular appearance, and show the presence of crystals. The location and size of these crystals become more distinct upon further treatment of the mount with 1% chromic acid solution. Then they are seen to be of considerable size in some grains, and often to be arranged in a group with their longer axes radiating from the hilum. Quite large crystals occur toward the outer portion of each layer, and their presence accounts for the difference in refraction of the layers at this point, which produces the appearance of concentric rings. Fig 3.

In double or compound grains a crystalline mass is seen at the center of each part, indicating that compound grains are formed by the deposition of material around a number of points of crystallization and the subsequent growth of each part until fusion occurs. In the outer layer of the grain no crystals appear, and this portion seems to be made up for the greater part of starch cellulose, which explains its lesser solubility in diastatic solutions. Fig 3.

An application of the differences in the structure of starch grains is of value in the examination of such closely related species as the common sarsaparillas of the genus Smilax. All the members of this group, which are commonly met with, have similar histological structures. All show the presence of raphides crystals of calcium oxalate and parenchyma cells filled with starch grains, which are commonly spherical, or united in groups of two or three grains. Honduras sarsaparilla, Smilax officinale, is characterized by starch grains varying from 7 to 20 microns in diameter, and raphides crystals 6 to 8 microns in length. The common Bamboo Brier root shows similar characteristics when examined in fine powder, but shows a variation in the starch grain. The starch grains of this drug greatly resemble those of Honduras sarsaparilla, being either single or united in groups, and show a similar structure in the interior of the grain; but upon measurement they range in size from 9 to 40 microns, averaging 29 microns in diameter. Hence, this means may be used in the differentiation of these plants when the drug is examined as a fine powder.

By the application of the above or of similar methods, it is possible to differentiate between very closely related plants. As our knowledge of the structure of starch grains is more fully developed, their value in the differentiation of such closely related species becomes apparent, and they are recognized as one of the greatest aids in Pharmacognosy.

*Botanical Department.*

# THE INLFLUENCE OF TEMPERATURE ON THE RESULTS SECURED BY HOUGHTON'S METHOD OF ASSAY FOR THE HEART TONICS.

CHAS. C. HASKELL, A. B., M. D.

From work carried out in this laboratory, it has been shown that temperature is an important factor when strophanthus preparations are assayed by Cushny's frog heart method, but apparently is of much less importance in regard to other members of the digitalis series. It seemed that temperature might similarly affect Houghton's method and the following experiments were carried out to settle this question.

A solution of ouabain, 1:1000 in 70 per cent. alcohol was used throughout as a representative strophanthus preparation, and also to ascertain whether it might serve as a standard. This solution was diluted to a strength of 1:20000 ouabain in 25 per cent. alcohol as needed for the assays. This diluted solution is called "Ouabain W."

As a representative digitalis preparation, a tincture was made from imported German leaf, 50 per cent. alcohol being used as a menstruum. This tincture was diluted with an equal volume of distilled water, reducing the alcoholic content to 25 per cent or less. In a later series, a tincture made from the same leaf with 75 per cent. alcohol was used, the dilution reducing the alcoholic content to about 30 per cent. These tinctures are designated A (50%) and B (75%).

In the first experiments, the frogs were injected and placed in small galvanized wire cages. Those kept at lower temperatures were placed in an insulated metal box, through which water circulated, while others were placed in an ordinary bacteriological incubator. The results secured in these experiments are given in Table I.

The question arose as to whether the conditions in the incubator other than the elevated temperature might not influence the frogs. To decide this, a series of assays were made in which the frogs were placed in cages in a large, over heated room. Needless to say, all the animals were kept moistened, there being about one-eighth of an inch of water in the bottom of each cage.

## TABLE I.

### OUABAIN W.

| Temp. 15-18° | | Temp 30-33° | |
|---|---|---|---|
| Survived | Died | Survived | Died |
| 0.00000030 | 0.00000040 | 0.00000020 | 0.00000020 |
| 0.00000040 | 0.00000045 | 0.00000020 | 0.00000025 |
| 0.00000040 | 0.00000045 | 0.00000025 | 0.00000025 |
| 0.00000040 | 0.00000050 | 0.00000025 | 0.00000030 |
| 0.00000045 | | | 0.00000030 |
| 0.00000050 | | | 0.00000030 |
| | | | 0.00000030 |

| | | | |
|---|---|---|---|
| 0.00000040 | 0.00000045 | | 0.00000022 |
| 0.00000040 | 0.00000045 | | 0.00000022 |
| 0.00000040 | 0.00000050 | | 0.00000022 |
| 0.00000040 | | | 0.00000022 |
| 0.00000045 | | | 0.00000020 |
| | | | 0.00000020 |

### Tr. DIGITALIS A.

| Temp. 15-18° | | Temp. 30-33° | |
|---|---|---|---|
| Survived | Died | Survived | Died |
| 0.0040 | 0.005 | 0.0040 | 0.0050 |
| 0.0050 | 0.006 | 0.0040 | 0.0050 |
| 0.0050 | 0.006 | 0.0055 | 0·0055 |
| 0.0060 | | 0.0055 | 0.0055 |
| 0.0060 | | 0.0055 | 0.0060 |
| 0.0070 | | 0.0060 | 0.0060 |
| 0.0070 | | | |
| 0.0070 | | | |
| 0.0070 | | | |
| 0.0080 | | | |
| 0.0080 | | | |
| 0.0090 | | | |

### Tr. DIGITALIS B.

| | | | |
|---|---|---|---|
| 0.011 | 0.011 | 0.006 | 0.0060 |
| 0.012 | 0.011 | 0.006 | 0.0060 |
| 0.013 | 0.012 | | 0.0060 |
| | 0.012 | | 0.0060 |
| | 0.012 | | 0.0065 |
| | 0.012 | | 0.0070 |
| | 0.013 | | 0.0070 |
| | | | 0.0070 |

## TABLE II.

| OUABAIN W. | | | | Tr. DIGITALIS B. | | | |
|---|---|---|---|---|---|---|---|
| Temp. 18° | | Temp. 25°-30° | | Temp. 18° | | Temp. 25°-30° | |
| Survived | Died | Survived | Died | Survived | Died | Survived | Died |
| 0.00000040 | 0.00000045 | 0.00000015 | 0.00000025 | 0.011 | 0.011 | 0.0060 | 0.0065 |
| 0.00000045 | 0.00000045 | 0.00000020 | 0.00000030 | 0.011 | 0.012 | 0.0060 | 0.0065 |
| 0,00000050 | 0.00000050 | 0.00000020 | 0.00000030 | 0.012 | 0.012 | 0.0065 | 0.0065 |
| | 0,00000050 | 0.00000020 | | 0,012 | 0.012 | 0.0065 | |
| | 0.00000050 | 0.00000025 | | 0.012 | 0.012 | 0.0070 | |
| | | 0.00000025 | | 0.012 | 0.013 | 0.0070 | |
| | | 0.00000025 | | 0.013 | | 0,0070 | |
| | | 0.00000030 | | | | | |

From these tables, it is evident that temperature markedly influences the resistance of frogs to poisoning by ouabain according to Houghton's method. The results with the tinctures of digitalis are not so clearly cut. At the higher temperatures, an estimation of the minimum lethal dose could be satisfactorily made; but when the temperature was reduced below 18°C the individual differences in resistance became more apparent. This may be explained by the fact that considerable variations in the lower temperatures possibly occurred during the night when the frogs were not observed, while no such variation took place in the automatically controlled high temperatures. As well as can be judged the resistance to ouabain and to the tinctures varies concomitantly.

*Department of Experimental Medicine.*

# THE COMPARATIVE ACTIVITY OF VARIOUS SPECIES AND VARIETIES OF DIGITALIS.

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[Presented to the Scientific Section of the American Pharmaceutical Association at Nashville August, 1913, and reprinted from the Journal of the American Pharmaceutical Association, March, 1914.]

But few of the many species and varieties of the genus Digitalis have been examined for comparative toxicity. Paschkis (1) claims that Digitalis ambigua contains the same constituents as Digitalis purpurea and that the medicinal properties of the two species are the same. Goldenberg (2) concludes that all species exert the same action on the heart of a frog as the infusion of Digitalis purpurea, but differ considerably in the energy of their action. He says that Digitalis eriostachys and Digitalis glandulosa are only slightly active and that Digitalis fontanesii is extremely feeble. Digitalis nervosa he finds to be 1.5 times and Digitalis ferruginea even 10 times more active than purpurea. This is indeed a small number of species upon which to formulate conclusions for a genus consisting of twenty-three or more species and numerous varieties. Boudgest (3) has examined Digitalis grandiflora which is synonymous with Digitalis ambigua and says that it possesses activity as a cardiac remedy which is fully equal to that of Digitalis purpurea.

This leaves a large number of species and horticultural varieties which have not been investigated for comparative medicinal value. For this reason as well as to gain more information upon the effects of cultivation, soils, geographical distribution and time of collection it was thought desirable to examine samples of as many species and varieties as could be obtained. Only samples from plants grown under uniform conditions and from seed of known source were used in the experiment. Catalogues from the most prominent seedsmen and nurserymen of the United States, England, Germany and Japan were examined and all the species and varieties listed by

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1 Paschkis: Apoth. Ztg., 1888, 869.

2 Goldenberg: Nouv. Remedes, Nov. 1893, 509.

3 Boudgest: Pharm. Ztg. 1903, 48, No. 92, from Nouv. Remed., 1903, No. 21.

them were obtained for planting. The first of these plantings were made as early as December, 1911, and the others extended through January, February, March and April of 1912.

All seeds were germinated in the greenhouse in seed pans filled with sterile soil. Other precautions were taken to guard against accidental mixture of seed. The small seedlings were transplanted to flats as soon as the second leaves had made their appearance. They were retained in the greenhouse in these plant flats until weather conditions would permit of their being transferred to cold-frames. Transplanting from the cold-frames to the open field commenced May 10th and continued without interruption until all plants were transferred. All varieties were grown in the same field upon a uniform soil consisting of clay loam. Cultivation was thorough, frequent, and continued throughout the growing season. With few exceptions the various forms were grown in uniform plots of one fortieth acre, requiring two hundred and sixty-four plants each. Leaves for the purpose of testing were collected seven months from the time of seed germination. The last collections were made September 19th. With two exceptions all samples were collected from non-flowering plants. The samples consisted of all the leaves from ten representative plants of each plot. These were dried either at room temperature or in a hot air oven at one hundred degrees centigrade. They were immediately reduced to a number sixty powder and sealed in amber bottles until tested. The table gives the species or variety, source of seed, date of germination, manner of curing, and effective dose as obtained by the frog-heart method of Cushny.

The table shows a variation of 84 per cent. in the effective dose as determined for the thirty-one samples. With few exceptions closely related forms show little uniformity in toxicity. The varieties which have originated from the species purpurea show a variation of over 75 per cent. Contrary to all expectations the highest value was obtained from lanata, a species quite distinct from purpurea. Gloxiniæflora alba a purpurea strain gave the lowest value. 83 per cent. of the total number of samples tested indicate a greater toxicity than the official purpurea. Compared with average digitalis leaf, 55 per cent of this number exceed the commercial article as indicated by effective dose.

With two exceptions all samples were collected from non-flowering plants. These exceptions were the synonymous forms, ambigua and macranthus, from which it was possible to collect samples from both the flowering and non-flowering plants.

ARRANGED ACCORDING TO NATURAL OR BOTANICAL RELATIONSHIP

| Species or Variety | Source of Seed | Germination | Manner of Drying | Effective Dose |
|---|---|---|---|---|
| Digitalis purpurea | Watkins & Simpson, England | 12- 1-11 | 100°C | 0.0009 |
| Digitalis purpurea | From wild plants, Oregon | 12-14-11 | Room temp. | 0.0009 |
| Digitalis purpurea alba | A. T. Boddington, New York | 12-15-12 | 100°C | 0.0005 |
| Digitalis purpurea rubra | A. T. Boddington, New York | 12-15-12 | Room temp. | 0.00045 |
| Digitalis purpurea rosea | A. T. Boddington, New York | 12-15-12 | Room temp. | 0.0004 |
| Digitalis gloxiniæflora purpurea | A. T. Boddington, New York | 2-10-12 | Room temp. | 0.00045 |
| Digitalis gloxiniæflora lilicina | A. T. Boddington, New York | 2-14-12 | 100°C | 0.0005 |
| Digitalis gloxiniæflora rosea | A. T. Boddington, New York | 2-15-12 | Room temp. | 0.0006 |
| Digitalis gloxiniæflora rosea | Henry A. Dreer, Philadelphia | 1-19-12 | 100°C | 0.0006 |
| Digitalis gloxiniæflora alba | A. T. Boddington, New York | 2-15-12 | Room temp. | 0.0019 |
| Digitalis alba | Watkins & Simpson, England | 12- 1-11 | 100°C | 0.0007 |
| Digitalis monstrosa | Watkins & Simpson, England | 2-15-12 | 100°C | 0.0008 |
| Digitalis monstrosa | Henry A. Dreer, Philadelphia | 2-15-12 | Room temp. | 0.00055 |
| Digitalis gloxinioides | Horsford's Nurseries, Vermont | 2-16-12 | Room temp. | 0.00045 |
| Digitalis maculata iveryana | A. T. Boddington, New York | 1-25-12 | 100°C | 0.0005 |
| Digitalis ivery's spotted | D. M. Ferry & Co., Michigan | 3-11-12 | 100°C | 0.00085 |
| Digitalis purpurea maculata superba | Ernst Benary, Germany | 4- 1-12 | Room temp. | 0.0005 |
| Digitalis mixed | J. A. Salzer, Wisconsin | 3-18-12 | 100°C | 0.00075 |
| Digitalis sp. | Yokohama Nursery Co., Japan | 3-18-12 | 100°C | 0.0009 |
| Digitalis sibirica | Horsford's Nurseries, Vermont | 12-28-11 | 100°C | 0.00055 |
| Digitalis buxbaumii | Ernst Benary, Germany | 4- 1-12 | Room temp. | 0.0005 |
| Digitalis gloxiniæflora lutea | A. T. Boddington, Vermont | 2-13-12 | 100°C | 0.0005 |
| Digitalis lutea | Ernst Benary, Germany | 4- 1-12 | 100°C | 0.00065 |
| Digitalis macranthus | Ernst Benary, Germany | 4- 1-12 | 100°C | 0.0008 |
| Digitalis macranthus (flowering) | Ernst Benary, Germany | 4- 1-12 | 100°C | 0.0005 |
| Digitalis ambigua | Horsford's Nurseries, Vermont | 12-29-11 | 100°C | 0.00055 |
| Digitalis ambigua (flowering) | Horsford's Nurseries, Vermont | 12-29-11 | 100°C | 0.0006 |
| Digitalis grandiflora | Henry A. Dreer, Philadelphia | 2-19-12 | 100°C | 0.0009 |
| Digitalis lanata | Horsford's Nurseries, Vermont | 12-28 11 | 100°C | 0.0004 |
| Digitalis lanata | A. T. Boddington, New York | 2-19-12 | 100°C | 0.0003 |
| Digitalis canariensis | Watkins & Simpson, England | 1-15-12 | 100°C | 0.0005 |

Macranthus shows a greater toxicity in the flowering stage while ambigua shows practically the same value for both the flowering and non-flowering plants. It is of interest to note that the species purpurea from England, Oregon and Japan have the same indicated value. The Japanese form which was supplied as Digitalis sp. proved upon flowering, to be one of the purest forms of Digitalis purpurea under investigation.

In this respect there is much uncertainty as to the nomenclature of the purpurea group and many of the varieties now recorded will doubtless be reduced to synonymy. Many of these varieties consist of a conglomeration of types which must be isolated and cultivated as pure strains before they will possess further value for experimental purposes.

The narrow leafed forms, represented by such species as canariensis, ambigua, lutea, and lanata have been found true to name and reproduce themselves in pure stand. Synonymy also exists in this group but the classification will be easier than in the broad leafed forms. A thorough systematic investigation of the whole genus will be necessary before further work can be done upon the comparative medicinal value of the various species and varieties.

In conclusion it may be pointed out that accurate botanical investigations should precede or accompany any comparative investigations of this group of plants; that the results indicate that good digitalis leaf may be obtained from the first year flowerless plants; that cultivation in itself does not lessen toxicity and that valuable forms other than purpurea may be found among the many species and horticultural varieties not hitherto investigated.

*Departments of Botany and Experimental Medicine.*

# THE INFLUENCE OF HEAT AND METHOD OF STORING UPON THE POTENCY OF DIGITALIS LEAVES

CHAS. C. HASKELL, A. B., M. D. AND F. A. MILLER, M. S.

Presented to the Scientific Section of the American Pharmaceuticial Association at Nashville, Aug. 1913, and reprinted from the Journal of the A. Ph. A., March 1914, p. 306.

Two points of much interest to the pharmacist and to the physician are the most desirable methods of curing and preserving digitalis leaves.

It is important to know whether the use of a moderately high degree of temperature is permissible, because, if such be the case, rapid drying can be accomplished and the theoretical, injurious enzyme destroyed and dependence upon favorable atmospheric conditions can thus be obviated.

Hart (3) finds that subjecting the leaves to an "excessive temperature" caused a loss in activity of twenty-five per cent. Unfortunately, he does not state what this temperature was. Hale (2) using dried leaves (moisture content of about 10 per cent.), concluded that the strength of the leaves, as determined by Cushny's method, is unaffected by a temperature up to 120°C. but that a temperature of 140°C. for two hours has an injurious action.

Our experiments were carried out upon leaves from conservatory grown plants of Digitalis gloxiniaeflora. These plants were eight months old when tested, and in some instances a sufficient supply of leaves was secured from each plant to enable us to carry out all comparative tests on a single plant, thus eliminating the possibility of variation due to individual plant differences.

The leaves, after drying, were reduced to No. 60 powder and tinctures made, using 75 per cent. alcohol as a menstruum. These tinctures were tested by Cushny's frog method and in a few cases the lethal dose for guinea pigs was also determined. In testing upon guinea pigs, the alcohol was not removed.

### EXPERIMENT I.

DIGITALIS GLOXINIAEFLORA, SINGLE PLANT

| No. | Dried at | Frog Assay | Guinea Pig Assay |
|---|---|---|---|
| B-1115 | 120°C. 4 hours | 0.0055 | 0.0020-0.0025 |
| B-1116 | 50°C. 24 hours | 0.0055 | 0.0030 |
| B-1117 | 35 C. 5 days<br>40°C. 3 hours | 0.0050 | 0.0025 |

## EXPERIMENT II.

### DIGITALIS GLOXINIAEFLORA, SINGLE PLANT

| No. | Dried at | Frog Assay |
|---|---|---|
| B-1124 | 120°C. 12 hours | 0.0065 |
| B-1128 | 80°C. 5 hours | 0.0065 |

The frog heart assays of these samples give values near those secured with similar samples of Digitalis purpurea. The extremely high toxicity for guinea pigs is explainable by the menstruum used. It has been our experience that an alcoholic menstruum of 75 per cent. enables the percolation of a tincture much more toxic for guinea pigs but not much stronger on frogs than one made with 50 per cent. alcohol.

From these experiments, it seems safe to conclude that a temperature of 120°C. applied to green leaves causes no greater loss in toxicity or ability to influence the frog's heart than does a temperature of 40°C.

There is apparently little agreement as to the keeping qualities of dried digitalis leaves exposed to air. Focke (1) claims that one month is sufficient time for a considerable loss in strength to occur when the leaves are exposed to the air and contain more than 1.5 per cent. moisture. Pratt (5) reaches somewhat similar conclusions. Hale, on the contrary, finds that leaves containing from 5.8 to 9.4 per cent. moisture are very active at the expiration of one, two, three and eight years respectively. Hatcher and Eggleston, (4) also, have been unable to see that digitalis leaves from three to five years old are any less potent than fresh samples.

Our own experiments bearing on this point have extended over only one year, but it seems that the additional evidence they present justifies their being reported.

The remainder of sample B-1124, which had been dried at 120°C. for 12 hours and gave a frog value of 0.0065 was divided into two portions, B-1129 and B-1130. These two portions were placed in wide mouthed amber bottles, one being carefully stoppered and sealed with paraffin, while the other was closed with a loosely-fitting plug of absorbent cotton.

## EXPERIMENT III

### DIGITALIS GLOXINIAEFLORA, SINGLE PLANT

| No. | Method of Storage | Original Assay | Assay 12 mo. later | Moisture |
|---|---|---|---|---|
| B-1129 | Open Bottle | 0.0065 | 0.0060 | 5.43 |
| B-1130 | Sealed Bottle | 0.0065 | 0.0060 | 2.31 |

The remainder of sample B-1128, dried at 80°C. for 5 hours which originally tested 0.0065 was divided into two portions, B-1131 and B-1132 and stored in a manner similiar to that described above.

### EXPERIMENT IV.

DIGITALIS GLOXINIAEFLORA, Single Plant

| No. | Manner of Storage | Original Assay | Assay 12 months later | Moisture Content |
|---|---|---|---|---|
| B-1131 | Open Bottle | 0.0065 | 0.0055 | 6.04 |
| B-1132 | Sealed Bottle | 0.0065 | 0.0060 | 2.83 |

A third sample was secured by collecting leaves from several different plants of Digitalis gloxiniaeflora. These leaves were dried at 30° to 55°C. for 20 hours, reduced to a No. 60 powder and gave a value of 0.006. Two lots were stored as in the preceding experiments.

### EXPERIMENT V.

DIGITALIS GLOXINIAEFLORA, Several Plants

| No. | Manner of Storage | Original Assay | Assay 12 months later | Moisture Content |
|---|---|---|---|---|
| B-1134 | Open Bottle | 0.006 | 0.006 | 5.92 |
| B-1133 | Sealed Bottle | 0.006 | 0.006 | 4.76 |

From these experiments, it is safe to conclude that in one year no deterioration occurred in our samples of leaf, irrespective of the manner of storage, even when the moisture content was as great as 6.04 per cent.

In some instances, a higher value was secured on the second assay than was obtained on the fresh leaf. This is to be explained by the limits of experimental error inherent in the assay method, as in all except one case, this difference did not amount to 10 per cent.

*Departments of Experimental Medicine and Botany.*

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SERIES 1 NUMBER 6

# THE LILLY SCIENTIFIC BULLETIN

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# THE PHYSIOLOGICAL ACTIVITY OF ERGOTIN AND POWDERED EXTRACT OF ERGOT

CHAS. C. HASKELL, A. B., M. D.

[Presented to the Scientific Section of the American Pharmaceutical Association at Nashville, Tenn., Aug 1913, and reprinted from the Journal of the A. Ph. A. June, 1914.]

There now seems little question as to the therapeutic value of the official fluid extract of ergot. It is sometimes desirable, however, to have solid or semi-solid ergot preparations in a more concentrated form than the crude drug, and it is also often advantageous to secure the active principles in a water-soluble form. These considerations, however, should have no weight, if it is learned that the process of manufacture of such special preparations destroys or greatly lessens the therapeutic activity originally possessed by the crude drug.

Two such preparations have recently been the subject of investigation in this laboratory and it seems of interest to report the results secured.

The first of these preparations is so-called Ergotin. It is made by treating powdered ergot with benzin to remove the oils, air-drying in order to free from benzin, and percolation with water. The percolate is reduced to a syrupy consistence at 90° to 120°C. and alcohol added. The resulting precipitate is removed by filtration and the filtrate reduced to a semi-solid extract at 90° to 120°C.

The second preparation is a powdered extract made by percolation of ground, benzin-extracted ergot with 50 per cent. alcohol; reduction of percolate to solid in vacuum dryer and addition of approximately an equal quantity of benzin-treated powdered ergot.

From theoretical considerations, it would seem that little if any ergotoxin would be contained in the ergotin and what activity it possesses would be due to the amines described by Barger, Dale, Kraft and others. The powdered extract, however, consists of about one-half powdered drug and the remainder of an alcoholic extract, so one would expect all the active constituents to be present, unless the benzin or the elevated temperature exercised a deleterious influence.

Ergot still finds its chief field of usefulness as an oxytocic, consequently, any laboratory attempt to estimate the therapeutic efficiency of the drug should be based upon a determination of its oxytocic power. It has been shown conclusively that a measurement of the pressor activity of ergot gives no reliable information of the value of the drug as a stimulant to uterine contractions, and it is illogical and useless to make such tests if the drug is to be used in obstetrical practice. It is not improbable, however, that blood pressure tests upon frogs may give reliable data as to the efficacy of the drug as a circulatory stimulant for man.

The excellent work of Edmunds and Hale has demonstrated that the cock's comb test gives results closely paralleling those secured when the drug is tested upon the uterus of an experimental animal. These authors point out that the cock's comb method is simple and allows of considerable accuracy.

During the past few months we have had occasion to examine twenty-four samples of crude ergot, four samples of ergotin, and nine samples of powdered extract of ergot. In every instance when a large enough dose was given intramuscularly to white leghorn cocks, bluing of the comb, diarrhea, and dyspnoea resulted, the symptoms produced by each preparation being practically identical. It seemed desirable, however, to test some of the samples of ergotin and powdered extract upon the uterus of a lower animal. Consequently, rabbits were used in the way described by Cushny, and a marked effect was produced by these preparations in every instance in which they were employed.

The tracings, Figures I and II, illustrate the results secured. They do not show the entire records as the uterus was allowed to return to and remain in a practically quiescent state for at least 1-2 hour before the drug was injected.

While feeling that blood pressure experiments give no positive information as to the value of the preparations as stimulants of the uterus, nevertheless a number of tests were made upon dogs, using the method of Wood and Hofer, except that 10 mgms. of morphine per kilogram were administered and the dogs also received a small dose of atropine, as suggested by Edmunds and Hale. The tracings, Figures III and IV show that these preparations have a marked pressor action.

From the evidence presented, it seems fair to assume that ergotin and powdered extract of ergot have the characteristic "ergot" action. It is of interest, however, to learn whether the full amount of drug activity is retained in these preparations.

In the manufacture of ergotin, approximately six pounds of drug are used to finish one pound of extract. For the powdered extract eight pounds of drug usually furnishes one pound of product. Consequently, ergotin should be six times and the powdered extract eight times as active as the crude drug.

Of the twenty-four samples of crude ergot examined by the cock's comb test, all were capable of causing bluing of the comb when given in a dose of 1 gram per kilogram. Of the nine samples of powdered extract examined, a dose of 0.25 gram per kilogram was efficient in all instances. Of the four samples of ergotin 0.25 gram per kilogram was efficient in two cases, while 0.3 gram was required in the other two. From this it seems that the powdered extract is about four times as active as the crude drug, while ergotin is, roughly about three and one-half times as active.

By blood-pressure experiments, however, a different relation is apparent. We have found that 0.1 c. c. per kilogram is the optimum dose of fluid extract, that is, this dose usually causes the maximal rise of pressure. With ergotin, however, the optimum dose seems 0.0125 gram per kilogram, or about 1-8 that of the fluid extract. Cronyn and Henderson have pointed out that large doses of ergot produce poor pressor response, and this is well illustrated by the tracings, Figures V, VI and VII, where it is seen that with the same preparation of Ergotin (391663) a small injection of 0.01 Gm. per kilogram sustains an increased blood pressure much better than does an injection of 0.025 or of 0.05 Gm. per kilogram. In fact the blood pressure 20 minutes after the injection of 0.05 Gm. per kilogram is actually below its initial pressure.

This may serve to explain the poor results that have been secured by others in testing the pressor action of these preparations.

In conclusion it may be said that:

1. Ergotin and powdered extract of ergot are capable of causing bluing of the cock's comb, contraction of the rabbit's uterus and rise of the dog's blood pressure.

2. There is apparently a considerable loss in the manufacture of these preparations of those substances influencing the uterine movements, while there is but slight loss in the pressor activity originally possessed by the crude ergot.

*Department of Experimental Medicine.*

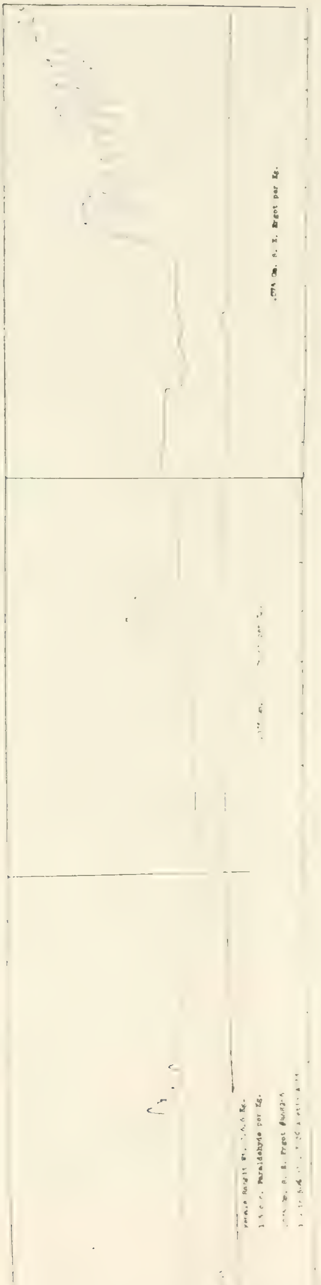

Figure I. Showing the contractions of a rabbit's uterus after the intravenous injection of Solid Extract of Ergot.

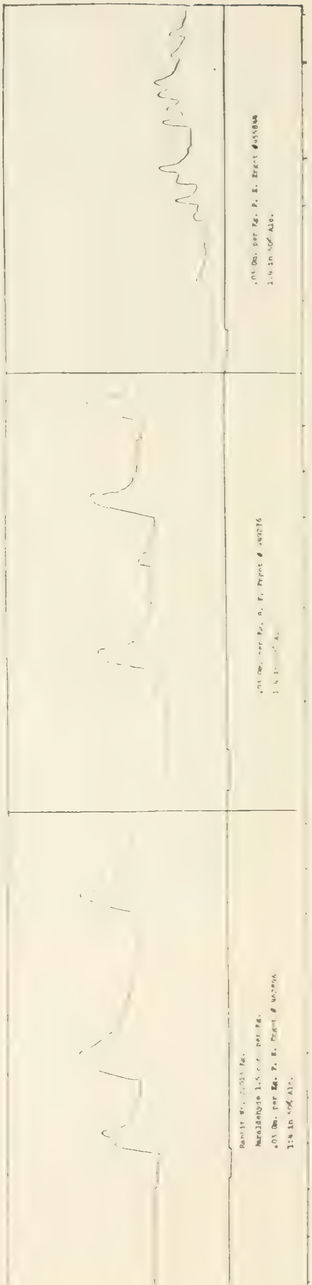

Figure II. Showing the contractions of a rabbit's uterus after the intravenous injection of P. E. Ergot and of S. E. Ergot.

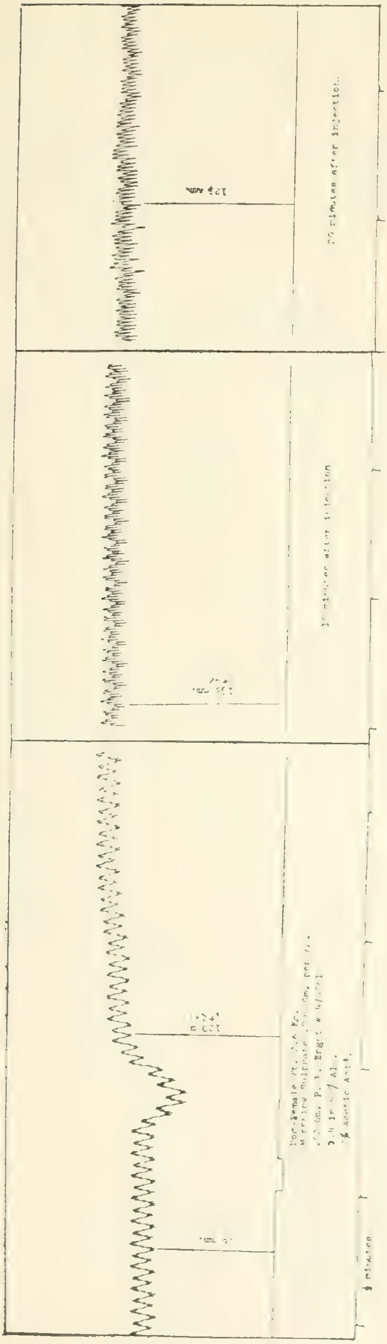

Figure III. Effect of Powd. Ext. Ergot on blood pressure of dog.

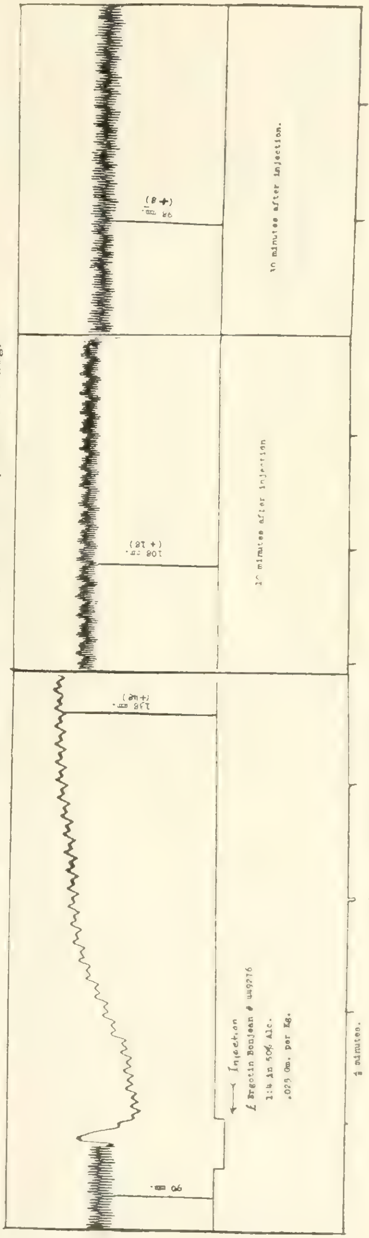

Figure IV. Effect of Ergotin, Bonjean, on blood pressure of dog.

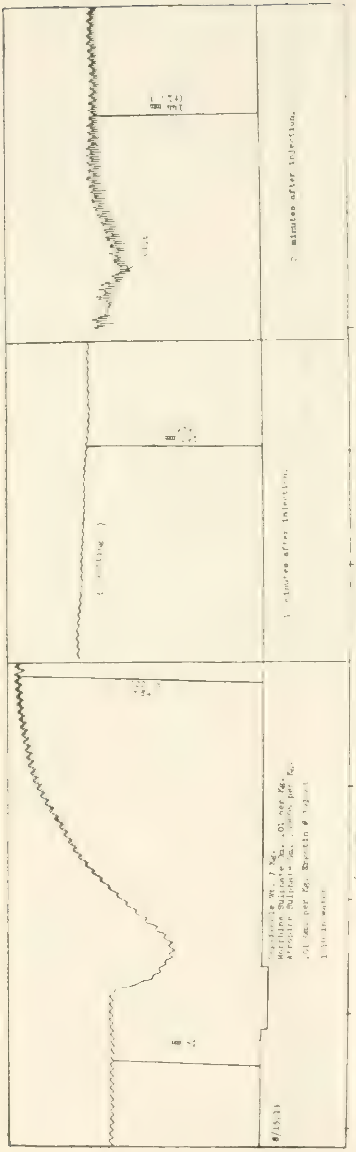

Figure V. Effect of Ergotin, Bonjean, in small amount on blood pressure of dog.

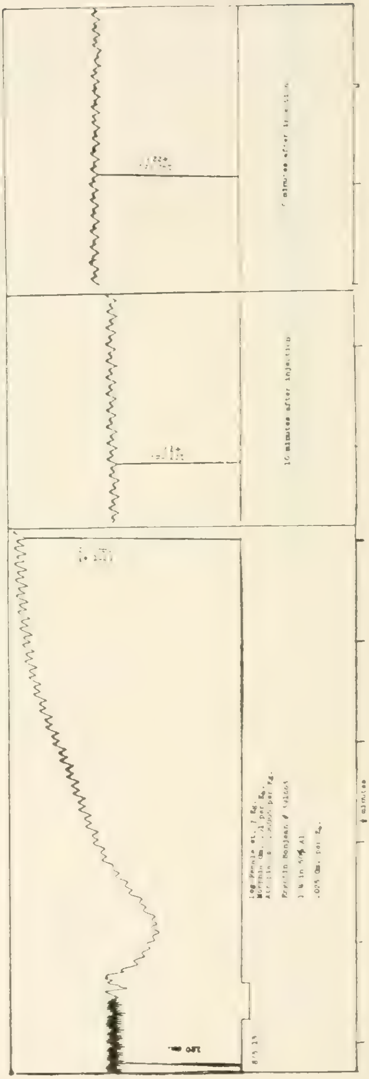

Figure VI. Effect of Ergotin, Bonjean, in medium amount on blood pressure of dog.

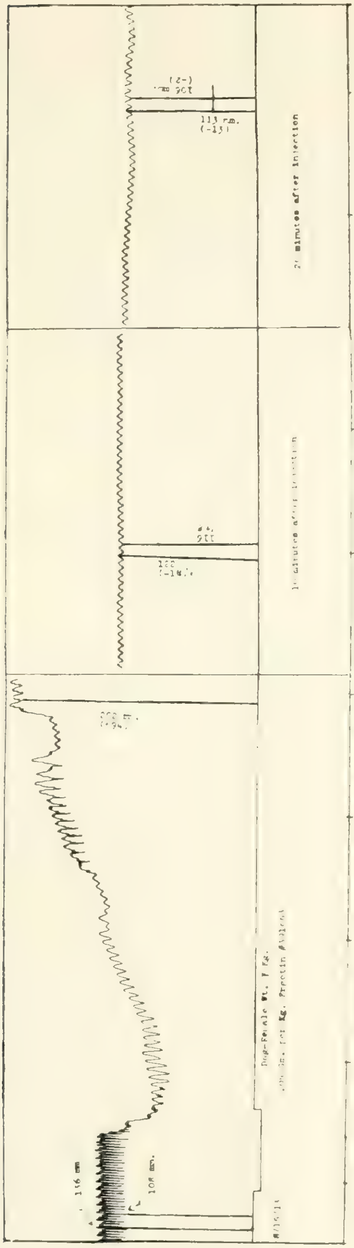

Figure VII. Effect of Ergotin, Bonjean, in large amount on blood pressure of dog.

# THE STABILITY OF OUABAIN IN AQUEOUS SOLUTION

CHAS. C. HASKELL AND W. A. DOEPPERS.

[Presented to the Scientific Section of the American Pharmaceutical Association at Nashville, Tenn., Aug. 1913, and reprinted from the Journal of the A. Ph. A. May 1914, p. 646.]

Galenical preparations and isolated pure principles of digitalis are comparatively slowly absorbed when administered by mouth to normal individuals. In patients suffering from cardiac disease, a condition of acute circulatory embarrassment often occurs, rendering it of the greatest importance to secure prompt drug action; but the venous stasis dependent upon the circulatory failure gives rise to engorgement of the gastro-intestinal mucosa and consequently, further delays absorption by this route.

In the treatment of such patients, clinicians have long desired some remedy that could be introduced directly into the blood stream and bring about full and prompt action. The infusion of digitalis has been employed in this way, but it would seem that it would be desirable to study upon lower animals the effect of introducing the number of undesirable constituents present in crude preparations of digitalis before the use of the infusion in this manner could be unqualifiedly endorsed. Then too, the experiments of Hatcher and Eggleston have shown that the commonly held opinion regarding the instability of the infusion is fully justified. Finally, it would be hard to secure an estimate of the strength of crude preparations of digitalis sufficiently accurate for use in standardizing remedies for intravenous use.

Credit is due to Hatcher for drawing attention to the possible value of strophanthin gratus, known as crystalline Strophanthin Thoms or better, as Ouabain. This substance is a crystalline glucoside, probably a definite chemical compound, isolated from Strophanthus gratus and is readily soluble in water or alcohol.

Amorphous strophanthin has been used quite extensively for intravenous administration and the results have often been very favorable. The chief disadvantage in the use of this substance is the uncertainty concerning its composition, it being probable that it is a mixture of different glucosides and different lots are apt to vary in strength.

Ouabain in a dry condition, kept under ordinary climatic conditions and exposed to air and light, undergoes no change in two years that can be detected by animal experiments. The convenience afforded by offering the physician a solution of ouabain in ampoules has led us to undertake a study of its keeping qualities when dissolved in solutions of different composition.

Ouabain was dissolved in distilled water, in normal salt (.9%) solution, in 5% alcohol, in 10% alcohol, in 25% alcohol and in 95% alcohol. The ouabain percentage was 1-10 and 1-100. Some of these solutions were tested upon frogs, using Cushny's one hour method, others upon guinea pigs, using a 24 hour lethal dose method, and a few upon cats, using Hatcher's method. The frog and cat methods have already been fully described elsewhere. The guinea pig method is a modification of Reed's. The dose is calculated per gram body weight and injected subcutaneously as in Reed's method. We have found it necessary, however, to take account of the alcoholic content of the solution and also advisable, always to use the same volume of fluid per gram, so 0.01 c.c. fluid per gram weight is always injected and 25% alcohol has been used in all the tests. It is also more satisfactory to observe the animal for 24 hours, because it is very common for pigs to survive more than three hours and succumb before the expiration of twenty-four hours. Occasionally an animal succumbs only at the end of forty-eight hours, but this is unusual. Attention has already been called to the unreliability of the guinea pig method in attempting to standardize the members of the digitalis group, but in a comparative study such as this the factor of seasonal variation does not enter. The results may be tabulated as follows: Drug strength means the strength of the original solution, which was reduced to 1:100000 for the cat assay, 1:200000 for the frog assay, and 1:100000 for the guinea pig assay whenever it was originally stronger. The figures under cat assay are fractions of a milligram per kilogram, under guinea pig and frog assay they are fractions of a gram per gram.

I. Ouabain in Amber Glass Containers.

| Age of Solution | Solvent | Drug Strength | Container | Cat Assay | Guinea Pig Assay | Frog Assay |
|---|---|---|---|---|---|---|
| 2 days | 95% Alcohol | 1:1000 | Flask | .0788 | .00000025 | .00000045 |
| 9 months | Water | 1.10000 | Amber Bottle | —— | .00000025 | .00000045 |
| 14 months | Water | 1.10000 | Amber Bottle | .0800 | .00000025 | .00000046 |
| 28 months | Normal Salt | 1.1000 | Amber Bottle | .0796 | .00000027 | .00000046 |

From these results it may be assumed that ouabain dissolved in distilled water or in normal salt solution and kept in amber bottles suffers no appreciable loss of strength in 28 months.

Two samples had been kept in glass-stoppered flint bottles and the assay of these gave rather surprising results.

II. Ouabain in Flint Glass Containers.

| Age of Solution | Solvent | Drug Strength | Container | Cat Assay | Guinea Pig Assay | Frog Assay |
|---|---|---|---|---|---|---|
| 2 days | 95% Alcohol | 1:1000 | Flask | .0788 | .00000025 | .00000045 |
| *22 months | Water | 1:10000 | Flint Bottle | —— | .00000036 | .00000067 |
| *32 months | Water | 1:1000 | Flint Bottle | —— | .00000055 | .00000075 |
| 36 months | 95% Alcohol | 1:1000 | Flint Bottle | —— | ——— | .00000053 |

*First and second assays on same sample.

From these tests, it seems that the nature of the container and the presence of alcohol influence the rate of deterioration. While an aqueous solution in an amber cork-stoppered bottle suffers little or no change in strength during twenty-eight months, an aqueous solution in a flint glass-stoppered bottle loses strength to a considerable degree in twenty-two months. Of course, too much weight should not be given the results obtained with such a limited number of samples.

Since the aqueous solutions of ouabain in amber bottles show no loss in strength that could be detected in 28 months, it seemed unnecessary at this time to test those containing alcohol, because other things being equal, an aqueous solution is preferable to an alcoholic solution for intramuscular or intravenous use. Frog assays upon these solutions showed that those containing 5 per cent. and 10 per cent. alcohol gave the same value at the end of 14 months as did the aqueous solutions, the containers being amber bottles.

In conclusion it may be said that the results of our work indicate that aqueous solutions of ouabain in amber glass containers are sufficiently stable to justify reliance being placed upon them by the physician.

*Department of Experimental Medicine.*

# THE INFLUENCE OF SOIL COMPOSITION ON MEDICINAL PLANTS.

F. A. MILLER, M. S.

[Presented to the Section on Scientific Papers of the American Pharmaceutical Association at the meeting held in Nashville, Tenn., Aug 1913. Rreprinted from the Journal American Pharmaceutical Association, March 1914, Vol. III, pp. 308-314 ]

Observations upon the influence of soil composition on medicinal plants have been made by several investigators. These observations have indicated that certain variations may occur in the percentage of active principles when the growing plants are subject to different soil conditions. Agriculturists have long recognized the influence of soil composition upon crop production. Their methods of measuring this influence, however, have differed from those employed with medicinal plants. Results have usually been determined by amount of total crop produced and not by chemical and biological assay. Recently some work has been done upon the chemical determination of certain constituents such as oil, sugar, and protein, as a means of following this influence. A combination of these methods would be essential to the best results with medicinal plants.

Hooper[1] demonstrated this fact by his work upon Jalap. He found that by using superphosphate at the rate of 1600 pounds per acre, he could bring about an increase in the dry weight of tubers produced as well as an increase in the percentage of resin. While the increase in resin by this treatment was only 24 per cent. the increase in dry weight was 86 per cent. Other investigators have given more attention to percentage of active principles than to crop production.

Carr[2] contends that although the addition of manure to a soil already suited to the growth of belladonna is no disadvantage and may be slightly beneficial, yet where large quantities of nitrogenous fertilizers are employed somewhat lower percentages of alkaloid are observed. This condition he attributes to the larger growth which results from such manuring. The author does not summarize his results with farmyard manure, nitrate, calcium cyanamide, basic slag, superphosphate, and potash. He concludes, however, by saying that in whatever latitude belladonna is grown, it will doubtless be found that the composition of the soil, the use of fertilizers, and seasonal conditions make for small variations. During the four years that he continued his experiment the results were not uniform according to the fertilizers used.

Ransom and Henderson[3] have studied the effects of kainit, superphosphate, sodium nitrate, potato mixture, basic slag, and combinations of these upon yield of green weight and percentage of total alkaloids in belladonna. In their conclusions they state that although they have not obtained sufficient evidence that the percentage of alkaloid in the dried leaf is materially altered by artificial manures, it would appear that in several cases the yield of green plant per acre has been largely increased. They further state that it would also appear that in the case of belladonna it is useless to hope that the drug shall possess anything approaching uniformity in medicinal potency, even when carefully collected and dried. It would seem that we are entirely too young in this work to make predictions of what the future may or may not disclose. The possibilities of obtaining uniformity in cultivated belladonna have hardly been touched upon and will not be known until the numerous problems of propagation and breeding have been thoroughly investigated. No efforts worthy of mention have as yet been made to produce a strain or variety of belladonna which will exhibit a uniform yield of alkaloids.

Chevalier[4] records data indicating an increase of nearly 60 per cent. of total alkaloids in belladonna when treated with nitrogenous fertilizer in combination with manure. Acid phosphate and potash produced no increase.

Tschirch[5] makes the broad, general statement that an increase in the amount of valuable constituents has been affected in the case of almost all cultivated plants.

To gain more information upon the influence of soil composition upon medicinal plants, experiments have been performed, using digitalis, stramonium, and belladonna. These experiments have been performed both in the open field and in the greenhouse with various commercial fertilizers and soils of different mechanical mixture. Precautions have been taken to secure uniform conditions in selecting locations of plots and in the collection and curing of samples.

## DIGITALIS

The plants for this experiment were grown from seed obtained in Oregon from wild plants. They were germinated December 8th, 1911, in the greenhouse, transplanted to flats and retained in the greenhouse until March 19th, 1912 when they were transferred to cold frames of double glass construction. Strong, hardy plants were thus obtained, eight hundred and seventy-five of which were used in the experiment. These were transplanted to the open field May 10th, 1912. Clean cultivation was practiced throughout the summer. The plot was located in an apple orchard between two rows of trees upon a soil which had been cropped to potatoes for several consecutive years without fertilization. The plot was divided into six equal parts and the fertilizers applied at the rate of six hundred pounds per acre. The nitrate of soda was divided into three equal portions and applied at intervals of two weeks. The fertilizers were not applied until the plants were well established.

They were then distributed by hand as uniformly as possible about the plants of each plot and immediately worked into the soil by cultivation. Mixed samples were collected for assay in early September, 1912 and cured at 100° centigrade. The following table gives the fertilizers used and the comparative results as obtained by the one hour frog heart method for assaying digitalis leaf.

| Plot | Sample | Fertilizer Used | Assay |
|---|---|---|---|
| 1 | B-1232 | Nitrate of Soda | 0.0065 |
| 2 | B-1233 | Sulphate of potash | 0.0062 |
| 3 | B-1234 | Normal Fertilizer | 0.0060 |
| 4 | B-1235 | Acid Phosphate | 0.0062 |
| 5 | B-1236 | Nitrate of soda<br>Sulphate of potash } equal parts | 0.0060 |
| 6 | B-1237 | Control | 0.0062 |

Little or no effect can be attributed to the use of these commercial fertilizers upon digitalis. This is evident from the fact that the difference in the comparative value of the samples from the different plots as indicated by the above figures is less than the possible experimental error.

## STRAMONIUM

The effects of a normal fertilizer upon the percentage of total alkaloids in stramonium when applied at the rate of 600 pounds per acre have been previously noted[6]. The average results obtained are repeated below.

DATURA STRAMONIUM

| No Fertilizer | Fertilizer | No Fertilizer | Fertilizer |
|---|---|---|---|
| 0.50% | 0.61% | 0.60% | 0.64% |

DATURA TATULA

| No Fertilizer | Fertilizer | No Fertilizer | Fertilizer |
|---|---|---|---|
| 0.49 | 0.54 | 0.62 | 0.68 |

## BELLADONNA

It is not only desirable to know the effects of commercial fertilizers upon medicinal plants but also the effects of various types and mixtures of soils. Only in this manner can the growth requirements of these plants be determined and their cultivation intelligently recommended. An open field experiment was performed upon first year belladonna plants using sodium nitrate, potassium sulphate, acid phosphate, and a normal fertilizer. The plants were started in the greenhouse March 8th, 1912, from seed taken from a commercial shipment of crude belladonna leaves, assaying 0.62 per cent. total alkaloids. All fertilizers were applied July 15th when the plants had reached a height of from twelve to twenty inches. They were applied by hand as uniformly as possible and immediately worked into the soil by cultivation. The total amount of sodium nitrate used was divided into three equal portions and applied at intervals of two weeks. All the fertilizers used were applied at the rate of six hundred pounds per acre. No commercial fertilizer other than stable manure had been used upon these plots for a period of at least twelve years. The samples for assay were collected September 19th, 1912 from representative plants of each plot. The following results were obtained:

FIRST EXPERIMENT

| | | | Assay | |
|---|---|---|---|---|
| Plot | Sample | Fertilizer | Leaves | Root |
| 1 | B-1258 | Sodium Nitrate | 0.699% | 0.510% |
| 2 | B-1259 | Potassium Sulphate | 0.858% | 0.504% |
| 3 | B-1261 | Acid Phosphate | 0.911% | 0.380% |
| 4 | B-1260 | Normal Fertilizer | 0.835% | 0.575% |
| 5 | B-1262 | Control | 0.688% | 0.420% |

The results of the first experiment indicate that marked changes may be produced in the percentage of alkaloids through the application of certain commercial fertilizers. However, sufficient data have not been obtained to justify any statement concerning the regularity of these changes. It is not known whether the changes indicated are due to the direct or indirect action of the fertilizers and what their influence would be when applied to different types of soils, under varied climatic and meteorological conditions. The extent of the error as introduced by individual plant variation in an experiment of this kind is also not known but must be considerable. Ten individual plants from this plot showed a variation of 50 per cent. True[7] in examining individual plants has found this variation to be as much as 70 per cent.

Disregarding this possible source of error, the increase in percentage of total alkaloids in the leaf as indicated by these trials is 20 per cent. for potassium sulphate, 24 per cent. for acid phosphate, and 18 per cent. for the normal fertilizer, the sodium nitrate causing no change. The increase in the root is 18 per cent. for sodium nitrate, 17 per cent. for potassium sulphate, 27 per cent. for the normal fertilizer and a decrease of 9 per cent. for acid phosphate.

To further test the effect of sodium nitrate and acid phosphate upon percentage of alkaloids another experiment has been performed upon second year plants. It was hoped in this experiment to eliminate the factor of individual plant variation in percentage of alkaloids. To accomplish this, fifteen uniform plants were selected and marked with conspicuous tags. These were divided into three groups of five plants each leaving considerable space between each of the three groups. All weeds were removed from around these plants and the soil thoroughly cultivated and pulverized. Before applying any fertilizer, samples were collected from the three groups of plants. In collecting these samples care was taken to obtain an equal number of leaves from each plant of a given group, and that these leaves should be of uniform size. This same procedure was followed in collecting the samples after the fertilizers had been applied. In this manner and by the utilization of the same plants throughout the experiment it is believed that the individual characteristics of the plants have been obviated and that the results should be more accurate than where no attention is given to the proportion of samples from different plants or to the utilization of identical plants throughout the experiment. After the original samples were collected plots number 1 and 2 were given a liberal application of sodium nitrate and acid phosphate respectively. Plot number 3 received no fertilizer. After an interval of two weeks plots 1 and 2 were given a second application. At the end of another two weeks' period samples were collected for assay in the manner designated. At this time the fifteen plants were removed from the soil and the principal roots from all plants of each group were taken for assay. At the beginning of the experiment a root sample was taken from three plants which were growing near the control group. Rain occurred after both applications of fertilizers thus insuring immediate availability.

SECOND EXPERIMENT

| | | Assay before Fertilizing | | Assay after Fertilizing | | |
|---|---|---|---|---|---|---|
| Plot | Fertilizer | Leaves | Root | Leaves | Increase in Leaf | Root |
| 1 | Potassium Nitrate | 0.558% | | 0.724% | 23% | 0.345% |
| 2 | Acid Phosphate | 0.569% | | 0.742% | 23% | 0.345% |
| 3 | Control | 0.598% | 0.385% | 0.724% | 18% | 0.322% |

The results indicate a considerable increase in the percentage of alkaloids in the leaf samples from all plots. The percentage increase in the fertilized plots is but little greater than that in the control. Practically no change is indicated in the alkaloidal content of the root samples.

The third experiment upon belladonna was carried on in the greenhouse with soils of widely different types. The plants used in the experiment were obtained by vegetative propagation from individuals of known alkaloidal yield. The individual plants were field-grown and were selected at random. Cuttings were made from them and placed in a propagating bed filled with sand. As soon as strong roots were developed the plants were potted to two inch pots, using a loam soil. They were later transferred to four inch pots. December 16th, 1912, they were removed from these and after all soil was carefully washed from the roots they were placed in six inch pots which had previously been filled with the different types of soils. In addition to the potted plants, ten were grown on a greenhouse bench filled with fibrous loam.

All the plants made very poor growth during the winter due to the low temperature of the greenhouse which was essential to the success of propagating work, being done at that time. The experiment was brought to a close September 7th, 1913, and on account of their small size it was necessary to use the entire plants for assaying. The proportions of root to stem and leaves were about the same in all cases. On account of the presence of roots in the assay samples the results would be expected to run lower than those of the original plants. The soils used in the experiments were as follows:

No. 1. From field in which original plants were grown.
No. 2. Equal parts clay and No. 1.
No. 3. " " " " sand.
No. 4. " " " " leaf mould.
No. 5. Leaf mould.

The clay was obtained from an excavation eight feet below the top soil. Sand was used in No. 3 to improve the physical condition of the clay. The leaf mould consisted of well decayed vegetable substance obtained from a woodlot of mixed growth.

The following table gives the number of the original plants from which the vegetative cuttings were made, soil and sample numbers, and percentage of total alkaloids as determined upon leaf samples from the original field-grown plants. All numbers of the same denomination in the first column refer to the same original plants from which the cuttings were made. A complete series from each of these original plants was started in the different soils but many of them were lost before the completion of the experiment.

The results indicate a considerable decrease in percentage of alkaloids in all plants examined, regardless of soil treatment. Even when grown upon the same soil as the original plant the decrease has been as great as from 20 per cent. in number 16, to 57 per cent. in number 10. Where two or more plants from the same individual parent were grown in the uniform soil on the greenhouse bench a variation is noted of from 9 per cent. in number 16, which is only slightly greater than the experimental error, to 20 per cent. in number 13. On the other hand, such widely different soils as number 1 and 4 produced a difference in alkaloidal yield of only 4 per cent. in plants from number 10, and 9 per cent. in those from number 17, and 8 per cent. on soils 3 and 5 in plants from number 13. After allowing 5 per cent. for experimental error there is little difference in favor of soil influence in the foregoing instances.

## THIRD EXPERIMENT

| Number | Soil Number | Sample Number | Assay | Original Plant |
|---|---|---|---|---|
| 10 | 1 | B-1664 | 0.351% | 0.820% |
| 10 | 2 | B-1665 | 0.373% | 0.820% |
| 10 | 4 | B-1666 | 0.364% | 0.820% |
| 13 | 3 | B-1667 | 0.351% | 0.870% |
| 13 | 4 | B-1668 | 0.382% | 0.870% |
| 13 | 5 | B-1669 | 0.323% | 0.870% |
| 13 | Bench | B-1670 | 9.498% | 0.870% |
| 13 | Bench | B-1671 | 0.396% | 0.870% |
| 13 | Bench | B-1672 | 0.477% | 0.870% |
| 14 | 1 | B-1673 | 0.441% | 0.664% |
| 14 | 2 | B-1674 | 0.365% | 0.664% |
| 14 | Bench | B-1676 | 0.383% | 0.664% |
| 15 | 1 | B-1677 | 0.283% | 0.600% |
| 15 | Bench | B-1678 | 0.471% | 0.600% |
| 15 | Bench | B-1679 | 0.501% | 0.600% |
| 15 | Bench | B-1680 | 0.442% | 0.600% |
| 16 | 1 | B-1681 | 0.412% | 0.516% |
| 16 | Bench | B-1682 | 0.398% | 0.516% |
| 16 | Bench | B-1683 | 0.436% | 0.516% |
| 17 | 1 | B-1684 | 0.454% | 0.616% |
| 17 | 2 | B-1685 | 0.404% | 0.616% |
| 17 | 3 | B-1686 | 0.370% | 0.616% |
| 17 | 4 | B-1687 | 0.412% | 0.616% |
| 17 | Bench | B-1688 | 0.373% | 0.616% |

The results as a whole indicate that further work is necessary on the influence of soil composition upon medicinal plants, before any generalizations can be made. The second experiment indicates that seasonal variations in alkaloidal percentage may have to be investigated more thoroughly. In this instance the percentage of alkaloids in the control plants increased nearly as much as in the fertilized plants. Also the influence of the two fertilizers, though apparently slight, seem to be identical with reference to the percentage of alkaloids. It is believed that there have been considerable sources of error in most of the work upon soils and fertilizers. It is desirable in this respect to first locate these sources of error and then attempt to eliminate them before proceeding further upon the problem of soil composition.

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# THE PROPAGATION OF MEDICINAL PLANTS

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An understanding of the best methods of propagating medicinal plants is essential to their successful cultivation. Their cultivation or commercial production is not a new industry, in fact certain phases of it are extremely old, though poorly developed. However long some of them have been continued under cultivation, there are still many phases of their propagation which have never been investigated. Repeated crop failures in the production of henbane in England have occurred during the past thirty years, and still the most recent reports continue to mention the uncertainty of germination without any attempt at explanation.

This condition is now changing, and the last few years have seen a more general and widespread interest in the thorough investigation of a rather selected list of drug plants than was hitherto experienced. The organized efforts of the United States Department of Agriculture are conducive to the continuation of this interest. England, Germany and Austria have not been slow in following the example of the United States, and are now advocating the national support of comprehensive drug plant investigations.

The success of these investigations must of necessity depend upon the readiness with which supplies of seeds and plants can be obtained, and the ease with which they may then be propagated. The means of propagating medicinal plants need not differ in the main from those employed for other economic forms. Seedsmen, nurserymen, florists and gardeners all have their special and well-perfected systems of propagation for their respective classes of plants. The questions involved in these systems are not only those for increasing the number of individual plants, but also those involving the blooming and fruiting periods, harvests, markets, special crops, improvements, and breeding operations. Any method of propagation which does not augment these conditions is soon abandoned. Also, in the case of medicinal forms, the problems of propagation, though essential of solution, are only supplementary to other more valuable and interesting ones. The improvement of drug plants through selective breeding and hybridization, their successful cultivation upon a commercial scale, with the involved questions of practical and economic production, are more attractive, but in the end are all dependent upon successful propagation.

With medicinal plants it is many times difficult to obtain sufficient materials from which to propagate. Isolated, restricted, and many times unknown regions of production, make it almost impossible to obtain either seeds or plants. Even syndicates and sometimes government control have so monopolized these regions that they are practically inaccessible to the individual investigator. In an effort to obtain sufficient material with which to evolve certain problems upon the cultivation and improvement of the more valuable drug plants, difficulties were soon encountered in locating reliable sources of supply. Any study of the methods of propagation must therefore consist of, first, the obtaining or locating of suitable materials and, second, the various methods by which these may be successfully propagated and perpetuated. The first part of this discussion will, therefore, deal with probable sources of seeds and plants and the second with their propagation.

A thorough search has been made in an effort to locate the best and most probable sources for these materials. The results are given under the following five divisions:

I. Commercial samples and shipments of crude drugs.
II. Crude drug merchants.
III. Individuals living in or near producing regions, or others carrying on investigations with similar plants.
IV. Public and private botanical gardens, experiment stations and other institutions.
V. Commercial seedsmen and nurserymen.

A discussion of these sources together with results obtained follows in the foregoing order.

## I.

From commercial samples and shipments of crude drugs in which the aerial parts of the plants are used, germinable seeds are often obtainable. Many lots of drugs must be examined, however, and large numbers of questionable seeds examined and tested. In addition to furnishing material for planting, this procedure also serves as a valuable means of accurately identifying some crude drug of questionable origin. The age of seed from this source is always unknown and considerable time must be allowed for possible failures in germination. Among the forms of which seed may be obtained in this manner are American wormseed (Chenopodium am-

brosioides), angelica (Angelica atropurpurea), anise (Pimpinella Anisum), arnica (Arnica montana), belladonna (Atropa Belladonna), broom tops (Cytisus scoparius) buchu (Barosma betulina) cannabis indica (Cannabis sativa), capsicum (Capsicum fastigiatum), caraway (Carum Carvi), cardamom (Elettaria Cardamomum), castor bean (Ricinus communis), celery seed (Apium graveolens), cevadilla (Schoenocaulon officinale), coca (Erythroxylon Coca), colchicum (Colchicum autumnale), colocynth (Citrullus Colocynthis), conium (Conium maculatum), coriander (Coriandrum sativum), fennel (Foeniculum vulgare), goat's rue (Galega officinalis), grains of paradise (Amomum Melegueta) henbane (Hyoscyamus niger), jambul seed (Abrus precatorius), larkspur (Delphinium Consolida), Levant wormseed (Artemisia pauciflora) lobelia (Lobelia inflata), nux vomica (Strychnos Nux-Vomica), parsley seed (Apium Petroselinum), passion flower (Passiflora incarnata), poppy heads (Papaver somniferum,) scullcap (Scutellaria lateriflora), senna (Cassia acutifolia and C. angustifolia), serpentaria (Aristolochia Serpentaria and A. reticulata), staphisagria (Delphinium Staphisagria), stramonium (Datura Stramonium).

Germination tests have been made upon a number of the above forms as follows:

| | |
|---|---|
| American wormseed | |
| Angelica | Fair germination. |
| Anise | |
| Arnica | Three tests. No results. |
| Belladonna | About 50 per cent. germination. |
| Broom tops | No results. |
| Buchu | 5 per cent. germination. |
| Cannabis indica | 100 per cent. germination. |
| Caraway | |
| Cardamom | Two tests. No results. |
| Celery seed | |
| Cevadilla | One test. About 25 per cent. |
| Coca | Four tests. No results. |
| Colchicum | Three tests. About 5 per cent. from one. |
| Colocynth | Most tests give from 5 to 10 per cent. germination. |
| Conium | 50 per cent. germination or over. |
| Coriander | |
| Fennel | |
| Goat's rue | One test gave 5 per cent. germination. |
| Grains of paradise | |
| Henbane | Most tests give 5 per cent. germination, or more. |
| Larkspur | 100 per cent. from most tests. |
| Levant wormseed | No results from 2 tests. |
| Lobelia | |
| Nux vomica | No results from 5 tests. |
| Parsley seed | |
| Passion flower | One test gave 4 per cent. germination. |
| Poppy heads | All tests gave 100 per cent. germination. |
| Scullcap | One test out of three gave about 5 per cent. germination. |
| Senna | Most tests gave fair germination. |
| Serpentaria | No results from two tests. |
| Staphisagria | One test gave 3 per cent. germination. |
| Stramonium | All tests gave 50 per cent. germination, or more |

Large numbers of seeds were used in most of these tests, and the conditions governing germination were varied to suit the requirements, in so far as they could be determined. It is to be noted that germination in most cases was low. Table IV gives details on most of these plants and on certain other miscellaneous drug plants.

## II

Dealers in crude drugs many times lend valuable assistance in searching for and supplying seeds and plants. Importers with foreign representatives and foreign merchants themselves will sometimes request their agents and collectors to assist in such matters. No great diversity of forms, however, can be secured through these sources. They are restricted to those of which the seed constitutes the medicinal part, or to those in which the seeds are likely to appear with other plant parts. The forms thus obtainable are mostly included in the preceding list, though better seeds are sometimes furnished by the dealers upon special request for fresh material.

## III

It is sometimes possible to locate individuals in different countries who have become interested in the collection of rare seeds and plants. Such sources are extremely questionable. While some individuals can furnish much good material, there are others who, though expressing much interest, cannot be depended upon to supply the proper species. Numerous instances of this have been recorded, an example of which may be noted where an enthusiastic druggist of Texas supplied fine specimens of Monarda for henbane, which he claimed was growing luxuriantly in his section of the state.

Local drug collectors may be considered here rather than with crude drug merchants. More strictly speaking, they are a class by themselves with many peculiarities and limitations. They are mostly illiterate persons, who take instructions poorly and disregard them when once in the field. A close personal association with many of them has revealed the fact that they are only capable of accurately identifying a very small number of medicinal plants. These usually occur among the monotypic genera and, when two or more closely related species are in question, are usually collected indiscriminately. One of the most intelligent collectors observed repeatedly brought in specimens of various species of Asclepias, upon requests and instructions for Apocynum cannabinum and Apocynum androsaemifolium. Apocynum cannabinum when finally located was supplied upon requests for both of the two species. Only recently a negro quack, who is held in high repute by the colored element of a considerable section, supplied Apocynum androsaemifolium upon request for Leptandra virginica. Another form supplied for Asclepias tuberosa will have to be grown to maturity before it can be identified. One collector was shown potted specimens of golden seal (Hydrastis canadensis), and stone root (Collinsonia canadensis), and failed to recognize either of them. Twin leaf (Jeffersonia diphylla) is repeatedly offered by dealers for genuine golden seal. Both the leaf and root structures of these forms are quite distinct.

## IV

In so far as their collections will permit, the botanical gardens and other similar institutions are of great value in supplying materials. Usually their collections of medicinal plants are comparatively small, but those which are obtainable are of high quality, and correctly classified. The liberal spirit of some of these stations is now restricted in some instances by government control. Such is the case with the buchu (Barosma betulina) of South Africa. To obtain seeds of this form direct from the Transvaal a long and tedious routine of grants, permits, etc., is necessary before they are allowed to be sent out of the country. On the other hand, such places as Jamaica Public Gardens, Jamaica, the Kew Royal Gardens near London, England, the Royal Botanic Garden of Calcutta, India, the Botanic Garden and Museum of Natural History of Para, Brazil, the Botanic Garden at Georgetown, British Guiana, and many others in foreign localities are usually willing to exchange or contribute materials for experimental purposes. Of no less distinction and willingness in this respect are the gardens at New York and St. Louis, the Office of Seed and Plant Introduction, and the Bureau of Plant Industry of the United States Department of Agriculture. However, it frequently happens that these excellent and widely separate stations fail to have the materials desired. At present, this condition exists with some unusually desirable species of the genus Artemisia.

## V

Commercial seedsmen and nurserymen have so far proven the best sources of supply, though they do not always maintain the highest standards of nomenclature. It is encouraging in this respect to note a growing tendency on their part to list more of their materials under the specific technical names. However, great numbers of varietal or trade names are annually introduced through these channels, which cause much confusion and uncertainty. Sooner or later many of these must be reduced to synonymy. Much time and energy is consumed in varietal tests for determining these synonyms, and any movement which will reduce their number must be greatly appreciated. What is most desired in this respect is the retaining of the native and introduced forms under their original names, or at least the recording of varieties in such a manner that the original parents can be traced, and the pedigrees of the supposed new forms accurately obtained.

The catalogues of the most prominent seedsmen and nurserymen from the United States, England, France, Germany, and Japan have been examined for medicinal plants. In such an examination it was necessary to have clearly in mind some means of deciding whether or not a given form should be classed medicinal or non-medicinal. Certain limitations must be exercised in such a distinction, and a conservative rather than a general attitude should be maintained. To follow the inclinations and suggestions of some authors would mean the consideration of an almost unlimited number of plants as possessing medicinal value. Practically, the list should not greatly exceed three hundred in number, and of these there are many included which are of doubtful value. In Bulletin No. 2, California State Board of Forestry, entitled "Pharmacal Plants and Their Culture," there is a context of one hundred and fifty-four pages, eight of which treat of the cultivation of medicinal plants in California. A portion of this small space is given up to such forms as sage, horehound, rosemary, marjoram, and dandelion. The greater bulk of the publication is taken up with a list of "Native and Introduced Medicinal and Poisonous Plants." The inclusion in this list of such forms as sugar maple, box elder, maidenhair fern, common edible mushroom (Agaricus campestris), wind flower, columbine, pawpaw, banana, garden beet, clematis, persimmon, California poppy, strawberry, holly, lemon, puffball, sorrel, cinquefoil, yucca and even corn, can hardly be explained. If such plants as the foregoing are to be considered medicinal, the list of three hundred would quickly grow to six hundred or more.

Based upon such a liberal classification, all seedsmen and nurserymen could be said to list medicinal plants regularly, and in large numbers, while as a matter of fact they list but very few. Certainly such a classification is not advisable, from either a practical or a scientific standpoint. The list which has been used as a means of dividing this material into medicinal and non-medicinal contains three hundred and eighty different forms, and includes all those that are commonly used by prominent manufacturers of pharmaceutical preparations.

### A LIST OF SEEDSMEN AND NURSERYMEN WHOSE CATALOGUES HAVE BEEN EXAMINED FOR MEDICINAL PLANTS, AND THE NUMBERS OF SUCH FORMS LISTED BY THEM

| Name of Firm | Location | Number Medicinal Forms Listed |
|---|---|---|
| Bash's Seed Store | Indianapolis, Ind. | 25 |
| P. J. Berckmans Co. | Augusta, Ga. | 20 |
| A. T. Boddington | New York City | 12 |
| Bobbink & Atkins | Rutherford, N. J. | 53 |
| Benary, Ernst | Erfurt, Germany | 68 |
| Biltmore Nursery | Biltmore, N. C. | 45 |
| W. Atlee Burpee & Co. | Philadelphia, Pa. | 18 |
| California Nursery Co. | Niles, Cal. | 26 |
| Childs, John L. | Floral Park, N. Y. | 17 |
| Dreer, Henry A. | Philadelphia, Pa. | 47 |
| Elliot Nursery | Pittsburgh, Pa. | 33 |
| Ferry, D. M., & Co. | Detroit, Mich. | 4 |
| Fottler, Fiske, Rawson Co. | Boston, Mass. | 17 |
| Gillett, Edward | Southwick, Mass. | 58 |
| Glen St. Mary Nurseries | Glen St. Mary, Fla. | 6 |
| Gregory & Son | Marblehead, Mass. | 3 |
| Isaac House & Son | Bristol, England | 5 |
| Peter Henderson & Co. | New York City | 21 |
| Horsford's Nurseries | Charlotte, Vt. | 35 |
| Haag & Schmidt | Erfurt, Germany | 158 |
| Kelway & Son | Langport, England | 10 |
| Medical Nursery | Calcutta, India | 50 |
| Livingston Seed Co. | Columbus, Ohio | 4 |
| C. C. Morse & Co. | San Francisco, Cal. | 8 |
| T. V. Munson & Son | Denison, Texas | 4 |
| Monroe Nursery Co. | Monroe, Mich. | 16 |
| Henry Mette | Quedlinburg, Germany | 58 |
| Oregon Nursery Co. | Salem, Ore. | 10 |
| Roumanille Lafayette pere & fils | St. Remy de Provence, France | 28 |
| John A. Salzer Seed Co. | La Crosse, Wisc. | 27 |
| J. M. Thorburn & Co. | New York City | 52 |
| Vaughan's Seed Store | Chicago, Ill. | 35 |
| Watkins & Simpson | London, England | 24 |
| J. D. Webster | Chichester, England | 15 |
| Yokohama Nursery Co. | Yokohama, Japan | 6 |

Based upon the number of important species listed, method and accuracy of nomenclature, class of material and general attitude the above list could be reduced to nine as follows: Haag & Schmidt, Benary, Mette, Medical Nursery, Gillett, Thorburn, Biltmore Nursery, Dreer, and Bobbink & Atkins. Materials have been obtained from five of these, and from eight of the others. The following discussion will consider not only the propagation of this material, but also such related problems as continued growth, productivity. commercial value, hardiness, habitat, and ease of transplanting, cultivating, and harvesting.

The methods of propagation have been those of standard use. Corbett[4] divides these methods into the natural and artificial. The natural methods include reproduction by seeds, spores, rootstocks, stolons, suckers or root sprouts, bulbs, corms, and tubers. The artificial methods consist in reproduction by cuttings, layering, grafting, and budding. Necessary adjuncts to all of these methods are suitable greenhouses, cold frames, hot beds, and open field accommodations.

Propagation from seeds has been practiced wherever possible, and must in nearly all cases precede other methods. Seed propagation, however practical it may be, will not serve all purposes. In breeding experiments and improvement selections, it is often very desirable to multiply individual specimens by other means. Inbreeding for the purpose of obtaining uniform strains is often a long and laborious method, while vegetative reproduction would give the same results in a shorter interval of time. Latent tendencies are more quickly and accurately eliminated, necessary seed production is avoided, as well as the saving of much time in seeding and transplanting.

Open field methods of seeding have been found impracticable and in many cases impossible with many medicinal forms. This condition necessitates a wide deviation from most normal methods of cropping and leads to the necessary development of specialized systems. Little of an agricultural nature has been developed with these forms. Farwell[8] says that belladonna, henbane, digitalis, and aconite can be grown as easily as potatoes. Turner[23] gives directions for growing henbane, which are too general to be of any value. Sander's[19] statements on the germination of seeds of medicinal plants are general, and include no methods or actual data. Henkel[10] says that digitalis offers no difficulties in cultivation, and advises sowing in the open field or in seed beds. True[22] advocates open field planting of digitalis. Kraemer[13] in his brief notice on seed germination, discourages open field sowing. Open field sowings of henbane, belladonna, and digitalis have been made for three successive years on well-prepared ground, and all have resulted in complete failures. Not enough plants for experimental purposes were obtained by the most careful attention.

Continued failures from open sowing of henbane are reported from England. Thirty years ago Holmes called attention to the difficulties in the cultivation of henbane. He mentioned the uncertainty of the crop in England, and the peculiarities in the germination of the seed. Again[11] in 1905 he mentions the uncertainty of the crop. Ransom[17] in 1902 claims that it is difficult to obtain a good crop of this drug in Britain. Henkel[10] later calls attention to the uncertainty in seed germination. Turner[23] in his directions for growing henbane is encouraging in the extreme. In his opinion it is not a difficult crop to handle. Repeated tests in the open field have resulted in failures. Fair germination was obtained in one instance, but the seedlings could not compete with common weeds until large enough for cultivation. Seed tests carried on under glass indicate that germination is of high percentage, uniform and fairly regular for seeds from different sources.

With some of the older drugs in cultivation, propagation and cultural systems have been highly developed. Examples of this may be found in the excellent notes of Weddell[24] on the nurseries, cultivation, harvesting and curing of coca, of Lumsdaine[15] on the cultivation of nutmegs and cloves in Bencoolen, of Eatwell[7] on the methods of cultivation, collecting, and curing of opium, and the influence of soil and climate upon the yield and quality of the product, and of DeVry[6] on cinchona.

There are few authentic data or directions, however, upon the propagation and cultivation of the more important forms which are adapted to this country. This list might well include such forms as belladonna, henbane, stramonium, digitalis, arnica, larkspur, valerian, santonica, veratrum, hellebore and others. It is evident that methods would have to be developed to suit the conditions of soil and climate, and at the same time be varied to meet the purposes of the work. That is, commercial production might be possible under a system which would be worthless for experiments on plant breeding and improvement.

In the following discussions the questions of propagation have been taken up in conjunction with other problems, and have only been developed as progress might demand. The work has been done for the purpose of determining the practicability of growing certain drug plants upon a commercial scale, and the possibility of improving them through an application of the methods of plant breeding. All seed tests have been of a practical nature, and but little of the great mass of material upon the effects of various reagents and influences upon germination has been used. This material so far seems to have resulted in no new general laws applicable to commercial growers. Experiments upon the effects of light and darkness (Heinricher[9]) upon seed germination, temperature (Reynolds[18]) in relation to germination, treatment with warm water (Jensen[12]), sterilization of soil (Stone & Smith[21]), copper sulphate treatment (Breal & Giustiniani[2]), electrical effects (Monahan[16]), soaking in chlorine water (Spatschil[20]), soil temperature (Brown[3]), action of ether and chloroform (Becquerel[1]), and delayed germination (Crocker[5]), have resulted in many data but few generalizations. Specific gravity tests, sterilized soils and the sulphuric acid treatment (Love & Leighty[14]), have been used successfully and might be recommended for practical purposes. The forms under consideration and the results so far obtained are as follows:

## DIGITALIS

More or less uncertainty exists as to the exact botanical source of this drug. The comparative medicinal value of the leaves from wild and cultivated forms, and the disputed methods of collection, curing and packing are unsettled questions of considerable importance. The comparative value of the many species and horticultural varieties, their ease of culture, relative yield of crude drug, their flowering periods, hardiness and duration of growth are additional problems which must be investigated before the genus can be made to yield its best to the manufacture of medicine.

Open field sowing was tried several times during two successive years. These tests were carried out both under practical field conditions and in ideally prepared seed beds. The results were complete failures and open seed sowing under field conditions cannot be recommended. After this preliminary work, the open field methods were abandoned for the more reliable greenhouse methods.

Seeds of twenty-seven varieties were obtained for experimental plantings. These were germinated (see Table I) in seed pans under glass, and transplanted to flats as soon as the second leaves were visible. These flats were retained in a cool greenhouse of 50 degrees night temperature, and 60 degrees day temperature, until March 1, when they were transferred to cold frames. The sashes of these frames were of double glass construction, and required little attention until the latter part of April. Then the sashes had to be removed during the day to prevent burning of the foliage, and to thoroughly harden off the young seedlings before transplanting to the field.

Transplanting to the open field was done mostly by inexperienced labor, and during dry weather accompanied by strong winds. The inexperienced labor was used as a means of determining the transplanting qualities of these forms, and the unfavorable weather only rendered this test more severe. After transplanting, the young plants were watered twice on consecutive days, and then left to the influence of natural conditions. Cultivation was commenced early, and repeated frequently throughout the growing season. The soil was a poor, stiff, clay loam, but all forms of the digitalis made excellent growth, as is evidenced by the amount of dry leaf produced by some average individual plants. Some of these yielded as follows:

Digitalis maculata Iveryana, a strong and robust form, 392 grams, Digitalis gloxinioides 170 grams, Digitalis Ivery's spotted 209 grams, and Digitalis alba 1,721 grams. In obtaining the comparative yield (see Table I) of all the varieties, ten average plants were selected, and all the leaves collected from them, and thoroughly air dried. The figures representing comparative yield of Digitalis alba, D. purpurea and D. canariensis, Watkins; D, sibirica, D. lanata, and D. ambigua, Horsford; and D. gloxiniaeflora rosea, Dreer, are all low on account of early collection. Those for the other forms, however, are accurate enough for all practical purposes. The comparative toxicity as given in the table is based upon figures obtained by the one hour frog heart method for testing Digitalis and its preparations. The value "one" represents the greatest toxicity, while the numerical increase indicates a proportional decrease in toxicity. Thus the weakest sample tested (Digitalis gloxiniaeflora alba) has a toxicity of 6.3.

Another object of the Digitalis study was to test the effects of hybridization upon medicinal value. For this work it was very desirable to bring the various species and varieties into flower as soon as possible. Early seed sowing augmented by a rather rapid growth in the greenhouse and by a slow hardy growth in the cold frames, as well as by a late and unusually favorable season, resulted in a number of flowering plants from several species and varieties (see Table II). For the purpose of obtaining early flowering plants upon which to begin the work of inbreeding and hybridization, a number of one year old plants were purchased from A. T. Boddington. These began flowering early, and furnished material throughout the season. Various combinations were tried, a number of which were successful, and seedlings are already being propagated in an effort to bring them into flower the first year. Thus any breeding operations can be annually continued or discontinued as the results may indicate, whereas these forms under the usual conditions of propagation would require two years to reach maturity.

It has also been noted that a small number of annual forms have appeared in the late plantings of several species and varieties, and especially where large numbers of plants were used. In all cases these have been inbred. The resulting progeny of these inbred annuals will be closely observed for the reappearance of annual forms.

Root division and propagation of lateral buds have both been tried with several varieties of Digitalis (see Table III). It was hoped that clonal varieties could be obtained in this manner. Uniformity of individuals and their behavior to varied soil and climatic conditions could then be studied with greater accuracy. Lateral buds, with and without roots, were carefully removed. Those removed during summer, fall and winter were extremely hard to start, whether they bore roots or not. Sand proved to be the best material for this purpose. However those made in early spring from plants left in the open have yielded a large percentage of plants. Side cuttings, with and without roots, made February 21, 1913, had rooted in sand in the greenhouse March 20, 1913.

## BELLADONNA

The propagation of belladonna was first attempted through open field sowing. Successive trials under varied field conditions with seed from several different sources resulted in complete failures. Fall sowing of imported seed upon a carefully prepared seed bed gave the following result. Seed planted September 3, 1912, fair germination October 21, 1912. Seed collected from growing plants August 15, 1912, planted September 3, 1912, fair germination October 21, 1912.

Greenhouse conditions were then tried, which were practically the same as those for digitalis, except that the seed pans used were more carefully protected from alternate degrees of moisture and dryness. See Table IV for germination data, and Tables V and VI for the influence of certain seed treatments upon germination.

The Belladonna seedlings transplant with considerable ease and with practically no loss. Damping off is not so frequent as with digitalis and henbane. Sterilized soil and careful watering greatly reduce this trouble in all cases. The belladonna seedlings were retained in the greenhouse in flats until well established, and were then transferred to cold frames. This was done on some occasions as early as March 19. Observations at this time showed a temperature of 46 degrees inside the frames, with the outside temperature below freezing. Outside night temperatures of 18 degrees caused no injury to the seedlings under the unprotected double glass sash. Seedlings transplanted directly into the frames in stiff clay soil made slower growth than those in the better soil of the flats, but produced hardier and more stalky plants, which transplanted to the open with much greater ease and certainty. They also made better subsequent growth than those in the flats. They required much less attention while in the frames, having to be watered only occasionally, while those in the flats dried out rapidly as the season advanced, and demanded considerable attention. Seedlings retained in the greenhouse in flats until May 10 were tender and succulent and wilted badly when transferred to the open field. From thirty to forty per cent. were lost during this second transplanting, while with those from the cold frames this loss was scarcely appreciable.

The successful propagation of the belladonna plant was only a necessary preliminary procedure to other more important problems. It was desired to observe this form under cultivation, to study its commercial production and the possibilities of developing a strain which would produce a uniformly higher yield of alkaloids than the ordinary commercial drug.

The first of these problems was accomplished through seed propagation. No difficulty was experienced in obtaining an abundance of plants after indoor methods of propagation were adopted. Large luxuriant plants were obtained from seed sown March 8, 1912, and were flowering by July 12. It is interesting to note here that the plants which had been transplanted directly from the seed pans to the poor soil in the cold frames were the first plants to commence flowering. The plants at this time (July 12) were from two to three feet tall, and much branched. Ransom[17] says that belladonna grown in England from seed sown in early spring produces little if any herb worth cutting the first year.

It is now believed that two profitable harvests can be obtained during one growing season, from plantings made January 1st of the same year. The belladonna plant is an herbaceous perennial, but on account of its susceptibility to winter killing in this climate, it may have to be grown as an annual. 1724 plants were left in the open without protection as a test for hardiness and as a means of obtaining individual plants of unusual hardiness from which to develop a strain more adapted to this climate. September 24 the entire herbaceous portion was removed from 1414 of these plants, the remaining 310 being left undisturbed. Twelve clumps of roots were lifted November 7, 1912, and placed in the cold frames with no protection except the double glass sash. The sash was put on at this time, and left closed until March 31, 1913, when the plants were first observed. At this time they were all bearing vigorous sprouts. Those left in the open were showing no growth on this date. These were again observed May 1, and the following conditions noted:

Number of plants, from which herbaceous portion had been removed, showing growth, 44.

Number of plants, which had been left undisturbed, showing growth, 307.

The second object of the belladonna investigation, viz: the attempt to produce a strain which would yield a uniformly higher percentage of alkaloids than the commercial drug, involves another method of propagation. In this case, individual plants must be examined for their property of yielding certain percentages of alkaloids. It is desirable to propagate promising individuals in such a manner that the high yielding character will be uniformly transmitted to the offsprings. As yet it is not known how such a character behaves. Alkaloids are not essential products of metabolism, and the inheritance of the property of producing a large percentage of such products is indeed questionable.

To investigate this point, both seed and vegetable propagation of pure strains must be practiced. The behavior of the character in these pure strains and their progeny will throw much light on the possibility of developing, propagating, and maintaining high yielding varieties through the practice of breeding.

If the property of an individual plant to produce a definite amount of alkaloids when grown under uniform conditions, behaves in the same manner as many external characters, the propagation of purebred strains by the vegetative method should yield uniformly according to selection.

To test this possibility, it was necessary to follow the methods of the florist in the propagation of cuttings in sand. This was done both in the greenhouse and in the open under cheese cloth shade. In the greenhouse the open sand bed was tried as well as the glass-covered bed recommended by Bailey. The open bed was also tried, with the cheese cloth shade. The best results in the greenhouse were obtained with the open bed under the influence of a mild bottom heat, and very careful watering. Sterile sand had to be used to prevent a total loss of the cuttings from the attack of a very small white worm, which fed on the lower ends of the cuttings, and prevented callousing until decay would commence. The best outdoor results were obtained from the use of the cold frames protected witn cheese-cloth shades. Top cuttings made from the more mature wood of the open grown plants strike root more readily than those from the tender succulent wood of the plants grown in the greenhouse.

Table III gives results obtained by the vegetative method of propagation for belladonna and other miscellaneous forms. Open field seeding has been found practical for such forms as stramonium (Datura Stramonium), cannabis indica (Cannabis sativa), larkspur (Delphinium Consolida), and lappa (Arctium Lappa). This list can probably be greatly enlarged as other forms are tested.

Various species and varieties of the genus Datura are being investigated in the same manner as belladonna and digitalis. Fifty individual plant assays have been made from plantings of Datura Stramonium and Datura Tatula. These indicate that the application of plant breeding methods to the improvement of medicinal plants may be successful, and extremely practicable. Averages of 50 per cent. and 60 per cent. have been obtained from the progeny of selected parent plants of Datura Stramonium and of 49 per cent. and 62 per cent. from Datura Tatula as compared with averages of 28 per cent. and 35 per cent. obtained respectively from wild plants of these two species. Individual plants have been found which assay as high as 65 per cent. for the D. Stramonium and 77 per cent. for D. Tatula. Such individuals are used for propagating purposes. Again, as in the case of belladonna, it is a question of inheritance that must determine the method of propagation or the advisability of attempting to perpetuate these high yielding individuals for commercial purposes.

In the foregoing, propagation has only been considered as a necessary preliminary procedure to other more comprehensive problems. A sufficient number of methods for propagating various classes of plants has been developed to suit the present need of medicinal plant growers. These have been largely the result of certain demands upon well-established horticultural forms, and their extended use is only a matter of application.

Their application herein described has furthered investigations not otherwise possible. The results obtained from their application, the continuation of other related problems, and the objects in view, have all been stated. The related problems such as commercial production, the testing of different species and varieties, and their improvement by breeding and selection, have an unquestionable economic value, the solution of which will lend much to the demands for better and more precise medicinal products.

TABLE I

FIELD RECORD

| Species or Varieties | Source | Date Planted | Date Germinated | Per Cent. Germinated | Date First Transplanting | Number Plants Used | Date Second Transplanting | Number transplanted to Field | Number at end of Season 10-31-12 | Samples Collected | Per Cent. Loss in Drying | Comparative Toxicity | Comparative Yield of Dry Drug |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1912 | 1912 | | 1912 | | 1912 | | | 1912 | | | gm. |
| *Digitalis purpurea rosea* | Boddington | 2-6 | 2-15 | 90 | 3-13 | 440 | 5-20 | 396 | 358 | 9-15 | 82 | 1.3 | 1,845 |
| *Digitalis purpurea rubra* | Boddington | 2-6 | 2-15 | 70 | 3-18 | 330 | 5-21 | 264 | 226 | 9-15 | 82 | 1.5 | 1,656 |
| *Digitalis lanata* | Boddington | 2-6 | 2-19 | 60 | 3-12 | 209 | 5-25 | 88 | 87 | 9-19 | 76 | 1.0 | 1,008 |
| *Digitalis* Ivery's spotted | Ferry | 2-29 | 3-11 | 60 | 3-29 | 330 | 5-24 | 264 | 244 | 10-11 | 86 | 1.8 | 2,090 |
| *Digitalis* mixed | Salzer | 3-7 | 3-18 | 90 | 4-2 | 330 | 5-25 | 176 | 153 | 10-18 | 82 | 2.5 | 1,755 |
| *Digitalis* sp. | Yokohama | 3-5 | 3-18 | 80 | 4-5 | 330 | 5-23 | 264 | 239 | 10-18 | 81 | 3.0 | 1,495 |
| *Digitalis lutea* | Benary | 3-22 | 4-1 | 60 | 4-11 | 330 | 5-22 | 264 | 256 | 11-1 | 70 | 2.1 | 554 |
| *Digitalis macranthus* | Benary | 3-22 | 4-1 | 70 | 4-11 | 330 | 5-23 | 264 | 231 | 11-1 | 67 | 2.6 | 430 |
| *Digitalis Buxbaumi* | Benary | 3-22 | 4-1 | 70 | 4-10 | 330 | 5-23 | 264 | 253 | 11-1 | 74 | 1.6 | 994 |
| *Digitalis purpurea maculata superba* | Benary | 3-22 | 4-1 | 60 | 4-11 | 330 | 5-25 | 176 | 168 | 11-1 | 77 | 1.6 | 1,360 |
| *Digitalis purpurea* | Oregon | 2-19 | 3-1 | 60 | 4-1 | 1390 | 5-10 | 1300 | 952 | 7-14 | 76 | 3.0 | 425 |
| | | 1911 | 1911 | | | | | | | | | | |
| *Digitalis alba* | Watkins | 11-22 | 12-1 | 90 | 1-30 | 345 | 5-20 | 264 | 193 | 7-25 | 78 | 2.3 | 391 |
| *Digitalis purpurea* | Watkins | | 12-1 | 90 | 1-30 | 351 | 5-25 | 88 | 83 | 7-25 | 80 | 3.0 | 312 |
| | | 1912 | 1912 | | | | | | | | | | |
| *Digitalis canariensis* | Watkins | 1-4 | 1-15 | 70 | 2-15 | 440 | 5-21 | 264 | 244 | 8-15 | 47 | 1.6 | 266 |
| *Digitalis monstrosa* | Watkins | 2-7 | 2-15 | 80 | 3-15 | 440 | 5-20 | 396 | 349 | 9-15 | 84 | 2.6 | 1,150 |
| *Digitalis sibirica* | Horsford | 2-8 | 2-19 | 80 | 3-11 | 220 | 5-21 | 396 | 380 | 7-28 | 72 | 1.8 | 149 |
| | | 1911 | 1911 | | | | | | | | | | |
| *Digitalis lanata* | Horsford | 12-20 | 12-28 | 40 | 2-6 | 99 | 5-25 | 176 | 173 | 7-28 | 76 | 1.3 | 219 |
| *Digitalis ambigua* | Horsford | 12-21 | 12-29 | 40 | 2-14 | 200 | 5-21 | 264 | 243 | 7-29 | 66 | 1.8 | 173 |
| | | 1912 | 1912 | | | | | | | | | | |
| *Digitalis gloxinioides* | Horsford | 2-7 | 2-16 | 80 | 3-4 | 450 | 5-20 | 616 | 552 | 9-16 | 82 | 1.5 | 1,850 |
| *Digitalis gloxiniaeflora rosea* | Dreer | 1-8 | 1-19 | 80 | 2-15 | 100 | 5-20 | 264 | 213 | 8-19 | 74 | 2.0 | 664 |
| *Digitalis monstrosa* | Dreer | 2-7 | 2-15 | 90 | 3-1 | 511 | 5-20 | 428 | 394 | 9-15 | 82 | 1.8 | 1,645 |
| *Digitalis grandiflora* | Dreer | 2-7 | 2-19 | 60 | 3-27 | 330 | 5-24 | 264 | 220 | 9-19 | 68 | 3.0 | 351 |
| *Digitalis maculata Iveryana* | Boddington | 1-17 | 1-25 | 80 | 2-13 | 333 | 5-21 | 264 | 228 | 8-35 | 84 | 1.6 | 1,050 |

### TABLE I (*Continued*)
### FIELD RECORD

| Species or Varities | Source | Date Planted | Date Germinated | Per Cent. Germinated | Date First Transplanting | Number Plants Used | Date Second Transplanting | Number Transplanted to Field | Number at end of Season 10-31-12 | Samples Collected | Per Cent. Loss in Drying | Comparative Toxicity | Comparative Yield of Dry Drug |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Digitalis gloxiniaeflora purpurea* | Boddington | 1–30 | 2–10 | 70 | 3–7 | 440 | 5–20 | 352 | 318 | 9–10 | 84 | 1.5 | 1,815 |
| *Digitalis gloxiniaeflora lutea* | Boddington | 1–30 | 2–13 | 60 | 3–6 | 440 | 5–22 | 264 | 262 | 9–13 | 74 | 1.6 | 882 |
| *Digitalis gloxiniaeflora lilicina* | Boddington | 2–6 | 2–14 | 80 | 3–8 | 440 | 5–22 | 308 | 272 | 9–14 | 80 | 1.6 | 1,715 |
| *Digitalis gloxiniaeflora alba* | Boddington | 2–6 | 2–15 | 80 | 3–16 | 330 | 5–24 | 308 | 224 | 9–15 | 82 | 6.3 | 1,475 |
| *Digitalis purpurea alba* | Boddington | 2–6 | 2–15 | 80 | 3–12 | 440 | 5–20 | 396 | 334 | 9–15 | 84 | 1.6 | 1,855 |

### TABLE II
### DATES OF FLOWERING AND NUMBER OF PLANTS IN FLOWER

| Variety | Source | Date Planted | July 15 | July 20 | July 25 | July 30 | Aug 10 | Aug. 10 | Sept. 1 | Sept. 20 | Oct 15 | Used |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Digitalis lanata* | Horsford | 1911<br>12–20 | | | | | | 1 | | 2 | 2 | 97 |
| *Digitalis ambigua* | Horsford | 12–21 | 1 | 17 | 35 | 49 | 54 | 87 | 151 | 217 | 220 | 233 |
| *Digitalis canariensis* | Watkins | 1912<br>1–4 | | | | | | | | | 2 | 224 |
| *Digitalis gloxiniaeflora lutea* | Boddington | 1–30 | | 1 | 1 | | | 3 | 11 | 68 | 95 | 386 |
| *Digitalis purpurea rosea* | Boddington | 2–6 | | | | | | 1 | | | | 358 |
| *Digitalis purpurea alba* | Boddington | 2–6 | | | | | 1 | | | | | 334 |
| *Digitalis gloxinioides* | Horsford | 2–7 | | | | | 4 | | 5 | 9 | 10 | 383 |
| *Digitalis monstrosa* | Watkins | 2–7 | | | | | 1 | 2 | | | 2 | 349 |
| *Digitalis grandiflora* | Dreer | 2–7 | | 1 | 2 | | 5 | 32 | 113 | 189 | 239 | 240 |
| *Digitalis sibirica* | Horsford | 2–8 | | | | | | | | 6 | 20 | 294 |
| *Digitalis* Iverys spotted | Ferry | 2–29 | | | | | | | | | 1 | 344 |
| *Digitalis* sp. | Yokohama | 3–5 | | | | | | | | | 5 | 239 |
| *Digitalis macranthus* | Benary | 3–22 | | 2 | 3 | | 16 | 81 | 161 | 217 | 222 | 231 |
| *Digitalis lutea* | Benary | 3–22 | | | | | | | | 1 | 3 | 256 |
| *Digitalis Buxbaumi* | Benary | 3–22 | | | | | | | 1 | 7 | 25 | 319 |

## TABLE III

### Propagation by Cuttings

| Name | Source | Number Started | Date Started | Number Rooted | Date Rooted | Method |
|---|---|---|---|---|---|---|
| | | | 1912 | | 1912 | |
| Belladonna | Field plants | 26 | 7–31 | 0 | | Greenhouse, open sand bed. |
| Belladonna | Field plants | 17 | 8–3 | 1 | 8–19 | Greenhouse, open sand bed. |
| Belladonna | Field plants | 24 | 8–1 | 0 | | Greenhouse, open sand bed. |
| Belladonna | Greenhouse plants | 76 | 5–14 | 73 | 6–6 | Greenhouse, bottom heat. |
| Belladonna | Greenhouse plants | 25 | 8–1 | 0 | | Greenhouse, bottom heat. |
| Belladonna | Greenhouse plants | 35 | 8–14 | 0 | | Greenhouse, bottom heat. |
| Coleus control | Greenhouse plants | 6 | 8–14 | 4 | 8–26 | Greenhouse, bottom heat. |
| Coleus control | Greenhouse plants | 19 | 8–22 | 0 | | Greenhouse, bottom heat. |
| Coleus control | Greenhouse plants | 28 | 8–22 | 0 | | Greenhouse, bottom heat. |
| Coleus control | Greenhouse plants | 5 | 1–30 | 0 | | Greenhouse, bottom heat. |
| Coleus control | Greenhouse plants | 121 | 1–22 | 0 | | Greenhouse, glass covered bed. |
| Coleus control | Greenhouse plants | 49 | 1–29 | 0 | | Greenhouse, open sand bed. |
| Coleus control | Greenhouse plants | 42 | 1–29 | 0 | | Greenhouse, open sand bed. |
| Coleus control | Field plants | 220 | 9–4 | 107 | | Cold frames, cheese cloth shades. |
| Coleus control | Field plants | 180 | 9–6 | 101 | | Cold frames, cheese cloth shades. |
| *Digitalis canariensis* | Field plants | 50 | 6–10 | 19 | 6–29 | Greenhouse, open sand bed. |
| *Digitalis canariensis* | Field plants | 40 | 7–13 | 0 | | Greenhouse, cheese cloth shade. |
| *Digitalis canariensis* | Field plants | 21 | 7–31 | 0 | | Greenhouse, open sand bed. |
| *Cytisus scoparius* | Greenhouse plants | 20 | 5–8 | 12 | 6–29 | Greenhouse, open sand bed. |
| *Cytisus scoparius* | Greenhouse plants | 55 | 6–29 | 30 | 8–30 | Greenhouse, open sand bed. |
| Nasturtium control | Greenhouse plants | 7 | 6–29 | 6 | 7–8 | Greenhouse, open sand bed. |
| *Cereus Grandiflorus* | Crude drug | 36 | 6–5 | 18 | 6–29 | Greenhouse, open sand bed. |
| Marshmallow | Field plants | 11 | 7–31 | 11 | 8–19 | Greenhouse, open sand bed. |
| *Solanum Dulcamara* | Field plants | 7 | 8–5 | 4 | 8–30 | Greenhouse, open sand bed. |
| Vanilla | Greenhouse plants | 21 | 6–20 | 14 | 8–20 | Greenhouse, open sand bed. |
| *Digitalis* | Field plants | 6 | 2–21 | 4 | 3–18 | In sand under bell glass. |

## TABLE IV
### SEED GERMINATION

| Name | Source | Date Planted | Date Germinated | Germination | Conditions |
|---|---|---|---|---|---|
| *Atropa Belladonna* | Greenhouse | 12–21–11 | 1–24–12 | Good | Greenhouse |
| *Atropa Belladonna* | Comm. sample | 12–11–11 | 1–26–12 | Uneven—fair | Greenhouse |
| *Atropa Belladonna* | Greenhouse | 1– 4–12 | 2– 9 12 | Good | Greenhouse |
| *Atropa Belladonna* | Comm. Lot. | 2– 9–12 | 3– 1–12 | Fair | Greenhouse |
| *Atropa Belladonna* | Comm. Lot | 2–19–12 | 3– 8–12 | Poor | Greenhouse |
| *Atropa Belladonna* | Dept. Agri. | 1–10–12 | 2–20–12 | Very good | Greenhouse |
| *Atropa Belladonna* | Dept. Agri. | 2–20–12 | 3–10–12 | Fair | Greenhouse |
| *Atropa Belladonna* | Aug. & Geo. Fischer | 2–26–12 | 3–21–12 | Good | Greenhouse |
| *Atropa Belladonna* | Cult. plants | 12 24–12 | 12–20–12 | Good | Greenhouse |
| *Atropa Belladonna* | Haag & Schmidt | 2–22–13 | 3–23–13 | Poor | Greenhouse |
| *Atropa Belladonna* | Select plant | 2–25–13 | 3–19–13 | Poor | Greenhouse |
| *Atropa Belladonna* | Select plant | 2–25–13 | 3 19–13 | Poor | Greenhouse |
| *Atropa Belladonna* | Select plant | 2–25–13 | 3–19–13 | Poor | Greenhouse |
| *Atropa Belladonna* | Select plant | 2–25–13 | 3–27–13 | Poor | Greenhouse |
| *Atropa Belladonna* | Select plant | 2–25–13 | 3 27 13 | Poor | Greenhouse |
| *Atropa Belladonna* | Aug. & Geo. Fischer | 9– 3–13 | 10 21–12 | Fair | Open field |
| *Atropa Belladonna* | Cultivated plants | 9– 3–12 | 10 21–12 | Fair | Open field |
| *Nicandra physaloides* | Haag & Schmidt | 2–22–12 | 3– 7–12 | Good | Greenhouse |
| *Hyoscyamus niger* | Benary | 2–22–12 | | | |
| *Hyoscyamus niger* | Benary | 3–22–12 | 4– 3–12 | Fair | Greenhouse |
| *Hyoscyamus niger* | Aug. & Geo. Fischer | 3–22–12 | | | |
| *Hyoscyamus niger* | Aug. & Geo. Fischer | 2–12–12 | 2–23–12 | Good | Greenhouse |
| *Hyoscyamus niger* | Aug. & Geo. Fischer | 2– 7–12 | 2–14–12 | Good | Greenhouse |
| *Hyoscyamus niger* | Haag & Schmidt | 2–25–12 | | No result | Greenhouse |
| *Hyoscyamus niger* | Haag & Schmidt | 2– 7–12 | | No result | Greenhouse |
| *Hyoscyamus niger* | Yokohama, Japan | 2–25–12 | 3–20–12 | Fair | Greenhouse |
| *Hyoscyamus niger* | Yokohama, Japan | 3–14–13 | 4– 3–13 | Poor | |
| *Hyoscyamus* sp. | Watkins & Simpson | 5– 5–13 | | No result | Greenhouse |
| *Hyoscyamus* annual | Cultivated plant | 5– 3–13 | 3–27–13 | Good | Greenhouse |
| *Hyoscyamus orientalis* | Haag & Schmidt | 2 10–13 | 2–27–13 | Poor | Greenhouse |

TABLE IV (*Continued*)
SEED GERMINATION

| Name | Source | Date Planted | Date Germinated | Germination | Conditions |
|---|---|---|---|---|---|
| *Hyoscyamus muticus* | Wheeler & Son | 2–24–13 | 3– 8–13 | Fair | Greenhouse |
| *Hyoscyamus muticus* | Aug. & Geo. Fischer | 3–20–12 | | No result | Greenhouse |
| *Hyoscyamus* sp. | Vaughn | 4–15–10 | | No result | Greenhouse |
| *Glycyrrhiza glabra* | Haag & Schmidt | 4–30–12 | 5– 9–12 | Very poor | Greenhouse |
| *Glycyrrhiza glabra* | Haag & Schmidt | 12–24–12 | 1– 2–13 | Poor | Greenhouse |
| *Bryonia alba* | Haag & Schmidt | 12–24–12 | | No result | Greenhouse |
| *Bryonia dioica* | Haag & Schmidt | 12–24–12 | | No result | Greenhouse |
| *Cinchona officinalis* | Haag & Schmidt | 2– 8–13 | 3–28–13 | Poor | Leaf mould |
| *Cinchona Calisaya* | Haag & Schmidt | 2– 8–13 | | No result | Leaf mould |
| *Cinchona succirubra* | Haag & Schmidt | 2– 8–13 | | No result | Leaf mould |
| *Erythroxylon Coca* | Haag & Schmidt | 2– 8–13 | | No result | Leaf mould |
| *Veratrum album* | Haag & Schmidt | 2– 8–13 | | No result | Leaf mould |
| *Veratrum viride* | Haag & Schmidt | 2– 8–13 | | No result | Leaf mould |
| *Veratrum nigrum* | Haag & Schmidt | 2– 8–13 | | No result | Leaf mould |
| *Colchicum autumnale* | Haag & Schmidt | 2– 8–13 | | No result | Greenhouse |
| *Gentiana lutea* | Haag & Schmidt | 2– 8–13 | | No result | Greenhouse |
| *Laurus Cinnamomum* | Haag & Schmidt | 2– 8–13 | | No result | |
| *Santalum album* | Haag & Schmidt | 2–10–13 | | No result | |
| *Strychnos Nux-vomica* | Haag & Schmidt | 2–10–13 | | No result | |
| *Convolvulus Scammonia* | Haag & Schmidt | 2–10–13 | 2–18–13 | Poor | |
| *Citrullus Colocynthis* | Haag & Schmidt | 2–10–13 | 3–28–13 | Poor | |
| *Piper nigrum* | Haag & Schmidt | 2–10–13 | | No result | |
| *Quillaja Saponaria* | Haag & Schmidt | 2–10–13 | | No result | |
| *Scilla maritima* | Haag & Schmidt | 2–10–13 | | No result | |
| *Aloe ferox* | Haag & Schmidt | 2–10–13 | | No result | |
| *Archangelica officinalis* | Haag & Schmidt | 3–14–13 | | No result | Greenhouse |
| *Anthemis nobilis* | Haag & Schmidt | 3–14–13 | 3–19–13 | Good | Greenhouse |
| *Arnica montana* | Haag & Schmidt | 3–14–13 | 3–21–13 | Fair | Greenhouse |
| *Levisticum officinale* | Haag & Schmidt | 3–14–13 | | No result | Greenhouse |
| *Pyrethrum cinerarifolium* | Haag & Schmidt | 3–14–13 | 3–24–13 | Good | Greenhouse |
| *Carthamus tinctorius* | Haag & Schmidt | 3–14–13 | 3–18–13 | Good | Greenhouse |
| *Foeniculum vulgare* | Haag & Schmidt | 3–14–13 | 3–24–13 | Good | Greenhouse |

TABLE IV (*Continued*)
SEED GERMINATION

| Name | Source | Date Planted | Date Germinated | Germination | Conditions |
|---|---|---|---|---|---|
| *Elettaria Cardamomum* | Comm. Sample | 12–27–12 | | No result | Greenhouse |
| *Delphinium Staphisagria* | Comm. Sample | 12–27–12 | 1–20–13 | Poor | Greenhouse |
| *Grindelia robusta* | U. S. Dept. Agr. | 2–11–13 | 2–15–13 | Good | Greenhouse |
| Insect flowers | Comm. Lot | 2–11–13 | | No result | Greenhouse |
| *Strophanthus* sp | Comm. Lot | 2–19–13 | | No result | Greenhouse |
| *Strophanthus* sp | Comm. Lot | 2–19–13 | 3– 5–13 | Poor | Greenhouse |
| *Althaea officinalis* | U. S. Dept. Agr. | 2–22–13 | 3– 5–13 | Good | Greenhouse |
| *Berberis Aquifolium* | Commercial | 3–10–13 | 4– 3–13 | Fair | Greenhouse |
| *Conium* sp | Comm. Lot | 3– 5–13 | 3–20–13 | Good | Greenhouse |
| *Theobroma Cacao* | Jamaica | 3–14–13 | | No result | Greenhouse |
| *Valeriana officinalis* | Dreer | 3–27–13 | | Good | Greenhouse |
| *Apium Petroselinum* | Comm. Lot | 4– 4–13 | 4–17–13 | Good | Greenhouse |
| *Amomum Melegueta* | Comm. Lot | 4– 4–13 | | No result | Greenhouse |
| *Apium graveolens* | Comm. Lot | 4– 4–13 | 4–16–13 | Good | Greenhouse |
| *Coriandrum sativum* | Comm. Lot | 4– 4–13 | 4–18–13 | Good | Greenhouse |
| *Chenopodium ambrosioides* | Comm. Lot | 4– 4–13 | 4–21–13 | Good | Greenhouse |
| *Carum Carvi* | Comm. Lot | 4– 4–13 | 4–17–13 | Fair | Greenhouse |
| *Delphinium Staphisagria* | Comm. Lot | 4– 4–13 | | No result | Greenhouse |
| *Lobelia inflata* | Comm. Lot | 4– 4–13 | | No result | Greenhouse |
| *Foeniculum vulgare* | Comm. Lot | 4– 4–13 | | No result | Greenhouse |
| *Foeniculum vulgare* | Comm. Lot | 4– 4–13 | | No result | Greenhouse |
| *Schoenocaulon officinale* | Comm. Lot | 4– 4–13 | | No result | Greenhouse |
| *Pimpinella Anisum* | Comm. Lot | 4– 4–13 | | No result | Greenhouse |
| *Citrullus Colocynthis* | Comm. Lot | 5– 1–12 | | No result | Greenhouse |
| *Aristolochia Serpentaria* | Comm. Lot | 5– 2–12 | | No result | Greenhouse |
| *Schoenocaulon officinale* | Comm. Lot | 5– 8–12 | 7– 8–12 | Poor | Greenhouse |
| *Amomum Melegueta* | Comm. Lot | 5– 8–12 | 5–20–12 | Fair | Greenhouse |
| *Strophanthus* sp | Comm. Lot | 5– 8–12 | | | Greenhouse |
| *Datura ferox* | Haag & Schmidt | 5–28–12 | 6– 4–12 | Good | Greenhouse |
| *Strophanthus Kombe* | Comm. Sample | 6–18–12 | | | Greenhouse |
| *Strophanthus hispidus* | Comm. Sample | 6–18–12 | | | Greenhouse |
| *Citrullus Colocynthis* | Comm. Sample | 6–18–12 | 7– 8–12 | Poor | Greenhouse |

TABLE V
Effect of Seed and Soil Treatment on Germination

| Name | Date Planted | Date Germinated | Germination | Treatment |
|---|---|---|---|---|
| Belladonna...... | 2—6—12 | 3—2—12 | Good | Water |
| Belladonna...... | 2—5—12 | 3—5—12 | Good | Warm water |
| Belladonna...... | 2—7—12 | 2—26—12 | Very good | Warm water |
| Belladonna...... | 2—7—12 | 2—26—12 | Very good | Warm water |
| Belladonna...... | 2—5—12 | 3—25—12 | Good | Warm water |
| Belladonna...... | 2—6—12 | 3—2—12 | Poor | Untreated |

TABLE VI
Concentrated Sulphuric Acid Treatment for Belladonna

| | Planted | Germinated | No. Seedlings | Date | No. Seedlings | Date | No. Seedlings | Date | No. Seedlings | Date | No. Seedlings |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1913 | 1913 | | 1913 | | 1913 | | 1913 | | 1813 | |
| Untreated .. | 2–14 | | | 3–13 | 3 | 3–19 | 3 | 3–28 | 14 | 4–8 | 24 |
| Treated 5 mi. | 2–14 | 3–5 | 2 | 3–13 | 4 | 3–19 | 10 | 3–28 | 27 | 4–8 | 36 |
| Treat. 10 mi. | 2–14 | 3–5 | 3 | 3–13 | 11 | 3–19 | 46 | 3–28 | 125 | 4–8 | 138 |
| Treat. 15 mi. | 2–14 | | | 3–13 | 3 | 3–19 | 15 | 3–28 | 32 | 4–8 | 31 |

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# SOME FACTORS IN DRUG ABSORPTION IN FROGS

W. F. BAKER, M. S. M. D.

[Presented to the Scientific Section of the American Pharmaceutical Association at the Detroit Meeting, Aug. 26, 1914, and reprinted from the Journal of the American Pharmaceutical Association, April, 1915, Vol. IV, p. 443.]

Among the objections that have been urged against the frog-heart assay method for the digitalis bodies, the principal ones are the factor of absorption and the time limit. Of these, the factor of absorption is undoubtedly the more important, the time limit being dependent upon the rate of absorption.

The manner in which various fluids are absorbed from the lymph sac or the absorption prevented, has not been determined. Experiments and experience have demonstrated however, that the majority of healthy frogs will absorb from the ventral lymph sac, in a period of one hour, a quantity of fluid equal to about 0.015 c.c. per gram of body weight.

Focke has demonstrated that enough of the digitalis bodies may be absorbed in five to twenty minutes to produce the characteristic digitalis heart and has taken twenty minutes as the time limit for his method. Magnus, using another method, has extended the time to one-half hour, Famulener and Lyons to one hour and Houghton to twelve hours. Not infrequently when assaying preparations by the one hour method and by the twenty-four hour method, I have found that out of a series of ten apparently healthy frogs in not more than one or two frogs would the absorption be complete. At another time, using the same preparation or solution and frogs of the same lot and under apparently the same conditions, absorption would be complete in every instance. If this non-absorption was but rarely observed the cause might be attributed to that ever ready idiosyncrasy. Occurring as it does at times in nearly every member of a series, it appears to be more than an individual peculiarity, and becomes a factor of great importance in this assay method.

While poor absorption may be met with during any time of the year, it is especially likely to occur during the Spring and late Fall. Certain preparations are very prone to meet with poor absorption, as German digitalin, digitoxin and preparations containing acetic acid or glycerin.

In a series of experiments with strychnine and ouabain, frogs placed in these solutions quickly (3 to 15 minutes) absorbed enough of the drug through the skin to produce the typical poisoning. The same results were secured after pithing (brain only for strychnine—brain and cord for ouabain.) In another series in which the solutions were injected into the lymph sac after pithing, complete absorption had taken place within 60 minutes in some frogs, while with others there had been but little absorption. In another series of experiments, a flap was made from the skin of the abdomen, and the drug painted on the under surface of the flap, that is, the lining of the lymph sac, and in others painted on the surface of the abdominal muscles, care being taken to prevent the solution touching the skin. Typical poisoning was produced in all cases except with ouabain on the inner surface of the flap when in only two instances was the ouabain action observed in a series of twelve experiments. In still another series after pithing, the skin was removed from the hind legs and the frog suspended so that these were in solutions of strychnine or ouabain. The typical poisonings were produced. This did not occur with strychnine if a ligature was previously placed around the base ot the heart.

From these experiments it would appear that absorption, of these two drugs at least, may take place from the outer or inner surface of the skin or from the surface of the muscles. The mechanism of absorption or prevention of absorption is not under the control of the central nervous system.

Frogs kept in light appear to absorb better than those kept in the dark. Absorption occurs more readily at higher temperatures although at a temperature of 30°C for one hour absorption is not always complete.

It was suggested that perhaps the manner in which the frogs were kept might have something to do with the absorption. Several series of experiments were made upon lots of frogs by testing them as soon as they arrived and again after they had been kept for some weeks under uniform conditions. The main points brought out by this work were, that different lots of frogs varied in their susceptibility to ouabain and digitalis when first received, and some lots absorbed more readily than others.

To determine if the amount of moisture present in the frog influenced absorption, frogs were kept in dry cages until they had lost 10, 25 and 50 per cent. of their weight and then solutions were injected into the lymph sacs. It was found that these frogs absorbed even less readily than the controls kept in wet cages.

In another series of experiments it was found that .9 per cent. salt solution was more readily absorbed than distilled water and 25 per cent. alcohol more readily than the salt solution. 50, 75 and 90 per cent. alcohol is fairly well absorbed but not so readily as 25 per cent.

Frogs which are plainly diseased (red-leg disease) may absorb quite readily while others which, to all appearances are quite healthy, may not absorb at all. In two instances of this kind, I obtained roughly, 25 per cent. more fluid from the ventral lymph sac than I had injected. I believe, however, that the matter of absorption is largely due to the health of the frogs and the condition under which they are kept and if proper attention is paid to the handling, cleansing and storing of the frogs, but little difficulty will be experienced with poor absorption. If when assaying, all frogs are discarded which contain an excess of fluid in the lymph sac one hour after being injected, quite uniform and reliable results are obtained by the one hour frog-heart method.

*Department of Experimental Medicine.*

# A RECENT BUCHU ADULTERATION

### R. B. HARVEY

[Reprinted from the Journal of the American Pharmaceutical Association, October 1912]

Commercial lots of Buchu recently appearing on the market have been adulterated by the addition of small amounts of foreign leaves. Although present to the extent of only three or four per cent. the intense astringency and bitterness of the leaves of this new adulterant make it especially objectionable. As no flowers or other diagnostic features were found, the botanical source of the leaves has not been determined, but they are probably derived from some shrub growing in the same locality as Buchu.

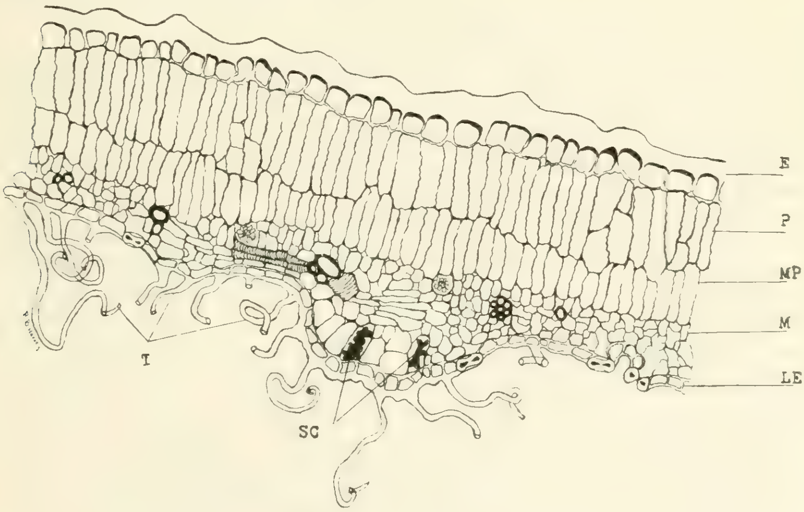

FIG. I.

The leaves of the adulterant are somewhat darker in color than Buchu and of a different shape. They are oblong-lanceolate, 10-20 mm. long and 3-8 mm. wide with acute apex and obtuse base. They are also much thicker than Buchu, the average being about 1-2 mm. The upper surface of the leaf is olive green, glabrous, and finely reticulate; the under surface, somewhat lighter in color and minutely tomentose. The margin is entire and revolute, and the texture coriaceous.

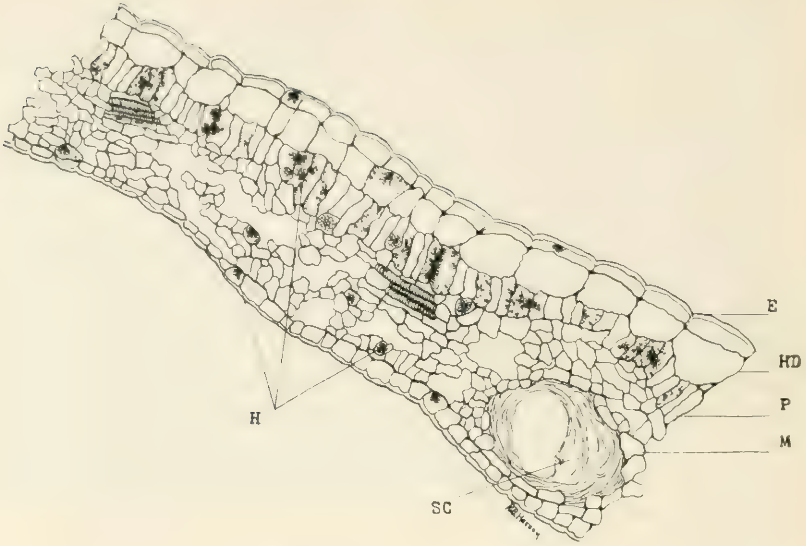

FIG. 2.

In cross section, the leaf of the adulterant (Fig. 1) shows a structure considerably different from that of Buchu (Fig. 2). The upper epidermis (E) of the adulterant is made up of thick-walled cells, the outer wall being 24-30 microns thick, striate, and unevenly papillate. No hypodermis corresponding to that of Buchu (HD) is found. The palisade layer (P) is made up of cells 90 microns long, and is followed by a layer of palisade-like mesophyl cells (MP) somewhat shorter than the first layer, and then by a mesophyl of spongy parenchyma (M). The lower epidermis (LE) is made up of comparatively small cells with moderately thickened walls. To this layer, longtubular and unicellular trichomes (T) are attached. These are intertwined and matted together and average 6 microns in diameter by about I-2 mm. long, A few brown secretion cells (SC) are found in the cross section but no hesperidin crystals (H) are shown. In powdered form the adulterant is detected by the upper epidermis which has much thicker walls than those of Buchu, and by the unicellular trichomes which are everywhere in evidence.

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# SCIENTIFIC BULLETIN

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# THE CULTIVATION OF MEDICINAL PLANTS

### F. A. MILLER, M. S.

[A lecture delivered to the Purdue Pharmaceutical Society at Lafayette and to the School of Pharmacy at Valparaiso University and reprinted from the Journal of the American Pharmaceutical Association, May, 1915.]

A brief historical review of the subject of drug cultivation in the United States reveals the fact that the subject is not a new one. It is only the revival of an old topic under modern ideas and more attractive possibilities. Concerning the introduction of this work in America, we would doubtless be safe in saying that the first medicinal plants to be cultivated were the so-called "kitchen herbs" in the gardens of the earliest settlers. As early as 1735 Collinson obtained over fifty kinds of "curious seeds" from the Chelsea Physic Garden of England, and Marshall, 1781, was one of the first to attempt the cultivation of the opium poppy in this country. Small gardens containing miscellaneous collections of plants, some of which might be classed as medicinal, were started in different localities only to be abandoned after a few years Most of these were established for educational purposes and some are still in existence. In these however, medicinal plants did not predominate, but were only supplementary to certain other families or groups of plants.

Not until the early part of the nineteenth century did medicinal plant-growing appear to have attracted sufficient attention to be considered as a commercial enterprise. The American Garden Calendar enumerates a large number of plants which could be grown in temperate climates and gives general directions for collecting and drying herbs, roots, seeds, etc. In this connection some of our present investigators of medicinal plants seem to be following this early example of McMahon in his Garden Calendar in still enumerating long tedious lists of what they call important medicinal plants and which they emphatically claim could be cultivated successfully in this country upon a commercial scale.

The most extensive operations ever carried on with drug producing plants in this country, were those of the Shakers. They began the work as early as 1800 at the original settlement at Mount Lebanon, New York. It seems they soon established quite a business in medicinal preparations from their cultivated drugs and the work was subsequently taken up by other Shaker colonies, particularly at Union Village, Ohio. Some idea of the amount of drugs produced by the Shakers as compared with the present demands may be gained from the statement that they produced at Lebanon as much as forty or fifty thousand pounds annually. This amount would about equal the average weekly demands of any one of the larger manufacturing concerns of today. While this movement spread somewhat in the Shaker community it never became very general. The Tilden Company, of the same locality, are said to have had as much as forty acres planted to medicinal plants. We find, however, that the work of Tilden & Co. was discontinued about 1880, and the operations of the Shakers gradually diminished until they finally attracted no more attention.

In times past the United States has shown itself capable of supplying materials, not otherwise produced here, which have been cut off by conditions of war. This has been true of opium, which was produced in considerable quantities during the Revolutionary War, the War of 1812 and the Civil War.

At the present time we are feeling the strain of similar conditions and find many of the most important European vegetable drugs not only cut off from the regular channels of trade, but also declared contraband of war. The list of drugs of strictly European origin which might be successfully cultivated in this country is not a large one, especially when we consider only those which are actually essential to the modern practice of medicine and those which from the nature of the plant we might reasonably expect could be grown and cultivated with some degree of certainty. In this list we have such drugs as aconite, belladonna, colchicum, convallaria, digitalis, gentian, henbane, opium, stramonium and valerian. Before seriously considering the cultivation of any of these, they must be studied carefully for peculiarities which may make it impossible to grow them successfully upon a commercial scale. The first thing to consider before deciding what forms one would attempt to grow is a reliable seed supply. This question apparently does not worry the prospective drug grower until he is actually ready to begin, and some have been known to purchase land, erect drying sheds, etc., before really knowing what they would grow, or where they could obtain seeds of the various medicinal forms.

In the above list, take for instance, aconite. Seeds of Aconitum napellus, the official species, may be obtained from several reliable seed firms of the United States. However, with good seed once in hand, we find that all difficulties are not immediately solved. The question at once arises as to how these should be sown. The average farmer or layman knows and considers but one method of seeding for commercial operations. This is the open field method and unless it can be practiced successfully there is little chance of rapid progress. Aconite is usually considered a tuberous rooted plant, with the tubers constituting the part used in medicine, the leaves being little used. These tubers do not reach maturity before the end of the second or third season so that when successfully seeded the plants must be grown through two or more successive years before a crop could be harvested. As to seeding we find that aconite is not easy to germinate even under the most accurately controlled conditions of a greenhouse. This eliminates field sowing and greatly increases the cost of production to say nothing of experienced labor and unusual equipment, such as greenhouses and hot beds. Aconite is often grown as a garden form, where its tall stately stems, surmounted by the spikes of dark blue flowers are both beautiful and interesting. This is no argument, however, that aconite would be an easy crop under field conditions. In this respect we find too many mistakes have already been made with medicinal plants. Many medicinal plants or their varieties have long been used as garden or decorative forms and upon this fact alone, sweeping statements have been made that they could be easily and economically produced upon a commercial scale. Even some college authorities who have strongly advocated drug cultivation have been accused of committing this error.

Whatever the sources of error, however, it is time for their correction as well as time for the adoption of more conservatism in the statements of those not informed upon the difficulties of commercial drug growing. To cite an example, a well-known writer in pharmaceutical circles, in giving directions for growing henbane states in extremely brief language that "culturally it could be associated with stramonium, cannabis indica, tobacco and belladonna." The absurdity of the association of such a group of plants from the standpoint of production is self-evident. A biennial, if we are to consider the pharmacopœial species of henbane, which reproduces from seed with much uncertainty, has given repeated crop failures in England and has been so difficult to handle in the United States that at one time the government, as well as individual workers, had given up their investigations of it, is here classed with a group of plants containing annuals, biennials and perennials, of widely different cultural requirements. Of this group, stramonium and cannabis are annuals of very easy culture, probably no easier one could have been selected than cannabis, and the difference between its cultural requirements and those of henbane could hardly be greater. In the case of tobacco we have a plant, old in cultivation, where all details of propagation, cultivation, and improvement have been highly perfected, as contrasted with henbane upon which practically nothing has been accomplished. Belladonna, the last named of the group with which henbane is associated, is a strict perennial of decidedly different requirements. It might be grown either as an annual, harvesting both leaves and roots at the end of each season, or left on the ground harvesting only the leaves annually. It requires a calcareous soil, and takes from four to six weeks for seed germination. We do not know the soil requirement of henbane and good seeds germinate in from six to eight days. As to the actual details of planting, cultivating, harvesting and curing, all of the above forms differ widely and no two of them could be handled by exactly the same method.

Returning to the plants under discussion we find that colchicum would give difficulties very similar to those of aconite. The parts used consist of the seed and the underground portion in the form of a corm. Seed may be obtained from manufacturers and crude drug dealers as well as from some European seed firms, but they appear to be very hard to germinate. I have tried many samples from the colchicum seed of the drug markets and one sample from Haag and Schmidt which was supposed to represent fresh seed, but so far have failed to germinate any of them. The seed has a very hard resistant seed-coat and it is possible that some special treatment may be necessary to facilitate more ready and uniform germination. This difficulty alone is sufficient reason for discouraging any attempt at commercial production without some investigations.

Convallaria, another one of the drugs mentioned, presents a different story. The rhizome is most commonly used and there is probably sufficient of the plant already under cultivation in this country to meet the usual demands for some time. Supplies of stock plants can easily be obtained and propagation from root cuttings offers no serious difficulties. Convallaria would be one of the easier medicinal forms to grow should such procedure become necessary.

In belladonna we find one of the most important of the group of vegetable drugs. The commercial supply of the crude drug has come almost entirely from central Europe. This source of supply is now completely shut off by existing conditions and previous crops have largely passed into the hands of manufacturers. As to the near future, the prospects of the drug being collected and marketed are extremely poor. Thus belladonna is one of the most promising medicinal plants from the standpoint of commercial supply and demand. To correct a common and rather widespread idea that this plant might be collected from the wild source in the United States, it should be clearly understood that belladonna (Atropa belladonna) does not occur in the wild state in this country in sufficient abundance for collection. The belladonna of the United States is nothing more than the little nightshade, Solanum nigrum, of common occurrence and which could in no manner serve as a substitute for the true European belladonna.

During the past ten years considerable knowledge has been gained on the cultivation of belladonna in the United States. The investigations of the Bureau of Plant Industry of the United States Department of Agriculture have furnished some valuable information upon the behavior of the alkaloids in the belladonna plant. In California the cultivation of belladonna has been fairly well established in certain localities, but the amount of marketable drug produced there has so far not been sufficient to decrease the annual demands for the foreign drug. The development of this industry has been slow and expensive and many times so discouraging as to almost cause its complete failure and subsequent abandonment. However, the present opportunity promises to be a great stimulus to these efforts and should go far toward seeing belladonna culture in this country, placed upon a sound commercial basis.

Viable seeds of the true pharmacopœial species of belladonna may now be obtained from several reliable sources in the United States in sufficient quantity for both experimental and commercial plantings. One of the most discouraging features of the cultivation of belladonna is the long period required for seed germination. This difficulty places the production of the plant outside of the class of regular farm operations and compels the grower to adopt methods with which he is not ordinarily familiar. When we consider that the seed requires from four to six weeks to germinate, even when under the influence of greenhouse conditions, the difficulties of field sowing are at once apparent. In the central states where weed growth on cultivated land is so luxuriant that almost constant cultivation is required, beginning a few days after the crop is sown, there would not even be a "struggle for existence" in the case of belladonna, for the simple reason that it would rarely come into existence in competition with our rapidly growing weeds. In some of the southern states where growing weather extends through from eight to ten months of the year and where the fall growth of weeds is not a serious factor, field sowing of the seed might be practiced with some degree of success. In our locality, however, experiments extending over several years have demonstrated that the only sure method of obtaining a good stand is by forced propagation and final transplanting to the open field. As to the rate at which plants may be handled by this method we find that an experienced man will move from the seed pans to plant flats or pots, from fifteen hundred to two thousand seedlings per day. This is when the man is working alone and doing all the preliminary work such as soil mixing, filling flats, and other details necessary to such operations. Transplanting to the open field is usually performed at the rate of one thousand to fifteen hundred plants per day. A good man with two helpers to space and drop the plants will work somewhat faster but his average will not greatly exceed fifteen hundred. Spacing the plants three by three feet in the field will require approximately forty seven hundred plants per acre. Only rough calculations are necessary to demonstrate the need of cheap but efficient labor in handling a crop of this kind. A mechanical device has been constructed, however, which by the use of three men, not, necessarily experienced in handling plants, and a two horse team, will transplant at the rate of from three to five acres per day, at the same time watering each plant and putting on an application of commercial fertilizer.

Everything is not accomplished, however, when the plants are successfully established in the field. In fact the work has only commenced. Subsequent cultivation for belladonna may be about the same as for corn until the plants begin to branch when some form of single plow must be adopted. In the meantime the crop must be protected from insects and other plant diseases. In this respect the problems involved are somewhat different from those of regular farm crops. In this particular instance both the leaves and roots of the plant are used. This means that any arsenical insecticide applied to the plant, must be used with great caution, or the arsenic, held mechanically upon the surface of the leaves and stems will eventually find its way into some finished product. However, if persistent efforts are made during the earliest stages of growth, the common potato beetle, which is probably the worst insect to contend with, can be eliminated for the entire season. Thus, by the time the plants have reached a size sufficiently large for collecting the first leaves of the young plants which may have received an application of insecticide have dropped from the stems. The safety of this procedure is evidenced by the free use of arsenicals on cabbage and other garden forms during the early stages of growth. Plant lice have also given serious trouble on belladonna but these can be controlled with any effective nicotine solution. The volatile nature of this substance makes it a perfectly harmless insecticide which may be freely used.

Having protected the plants from insects by some method and carried them safely through the growing season, the approach of harvest brings forth another series of problems. The first to suggest itself will have reference to the time of collection. When the final product is placed on the market its percentage of total alkaloids will be one of the important determining factors in judging the quality, and whether it is belladonna or some other form there will be some definite stage in the development of the plant when the yield of alkaloids or other active principles will be greatest. This condition should determine the time of collection and it can only be ascertained by the collection and testing of many series of samples.

Other questions which the grower would be compelled to answer would be the method of collection, for both leaves and roots and the manner of curing these when once they were successfully harvested.

To continue with the list of European drugs we find that it is the opinion of many practitioners that really active digitalis comes only from England, Germany, or some other source, known only to the man who compiles the information on the label. There is in Oregon and Washington an abundant growth of good digitalis which shows an equal value with either the German or English drug. This source of supply could easily take care of the entire demands of the United States. With this abundance of material within easy access it would not be necessary or wise to attempt the commercial cultivation of digitalis. Those wishing to do so may easily obtain seed of Digitalis purpurea from any reliable seed firm. It cannot be sown in the open field, as several writers have stated, but must be propagated about the same as belladonna. It is a hardier plant, however, and may be carried through the seedling stage at a somewhat lower temperature. It flourishes in the ordinary cold frames and may be placed in these with perfect safety as early as March 1st. Only the leaves of this plant are used. The present pharmacopoeia requires that these be collected from flowering plants. It is possible, however, that the revision committee will respond to the influence of recent research upon this subject and eliminate that part of the requirement which refers to the flowering plants. Digitalis being biennial, the present pharmacopoeial requirement makes it impossible to even think of growing the drug at a profit. Indeed it is doubtful whether it could be cultivated and harvested at the end of the first year with any hope of financial returns.

Gentian was another of the drugs mentioned and is one which holds a rather important place in medicine. It would be a promising drug plant to have established in this country, but we must begin at the beginning. Let us not prepare to grow gentian until we at least have gentian to grow. As in many other instances we find that we cannot obtain good seed of Gentiana lutea. I have had seed of this species from Erfurt, Germany, but it failed to germinate. Looking a little further into the history of the plant we find it to be a perennial of which only the roots are used. If we had an abundant supply of good seed with which to begin tomorrow, we could not under these conditions expect to harvest a crop at the end of the present season.

Concerning henbane it is also to be regretted that the United States is not now prepared to produce its own supply of this valuable drug. It is not, however, and the few individuals who have been attempting to grow it should not be discouraged when the government which is at present leading the movement in drug plant cultivation, has almost failed in its attempt to grow the plant. Good seed of henbane is difficult to obtain and I would not advise anyone to depend upon those which have not first been thoroughly tested. I have isolated an annual strain, the seed of which germinates quickly and uniformly. Several hundred plants of this strain will be grown this year. This should be a sufficiently large number of plants to test the strain for commercial purposes. The official biennial species has been hard to grow to maturity. Once this is accomplished and a good supply of fresh seed obtained the work on this form should progress more rapidly. One of the worst things to contend with in growing henbane, even on a small experimental scale, is the ravage of the potato beetle. It has been almost impossible to protect the plants from this insect even by the use of the most effective poison.

Concluding with stramonium we find that what was said of digitalis will apply in some degree to this form. With the wild plant growing abundantly in the United States, there is some question of the advisability of attempting its cultivation. It is usually supposed that stramonium will grow in spite of itself, but such is not the case and good results could only be expected on rich soil and by the practice of clean cultivation.

This leads us into a discussion of the problems of drug cultivation from a different viewpoint. We have too often heard of the fortunes to be derived from weeds, of the statements that this or that plant will grow and flourish anywhere and we as often wonder upon what type of reasoning such statements are founded. We know that history records but very, very few fortunes as having originated from weeds except probably through their elimination. It is also evident that all plants now known to man, do possess selective properties which enable them to reach their maximum development only under environmental conditions which are within certain definite limits. The mere fact that a form has been observed growing as a weed is not good evidence that it will behave in a similar manner when introduced into cultivation. In fact, this is not what we desire. If we merely wish to grow them as weeds and thereby realize an abundant profit we had better stop where we are now, for we would likely derive neither weeds nor profit. What is most desired is to grow medicinal plants not as weeds, but as cultivated plants, improved, perfected and developed in the same manner and to the same degree of perfection as already obtains in the case of other economic forms. To merely grow medicinal plants upon a commercial scale is not sufficient. In fact it is very doubtful whether or not most of them can be produced economically unless some improvement is followed. We cannot compete with European producers upon a mere basis of equality. We may be able to compete with them, however, by increasing the quality and yield of the drugs through improvement. This can only be brought about through careful breeding and selection.

The field of research in ths respect is almost unlimited and practically untouched. There is hardly a vegetable drug of any importance that does not suggest opportunity and the possibilities for improvement in this line of work. We only need turn to a careful consideration of existing conditions with reference to such drugs as henbane, belladonna, digitalis, stramonium, cannabis, and others for these suggestions. Henbane of disgraceful appearance and low in alkaloidal value is admitted to the United States in order to meet the constant demand for this drug. We have an official description of this drug and a species requirement, but who knows or is able to determine what species are actually included in the crude drug of European origin. From the appearances of many shipments it seems that every species, both plant and animal, which might inhabit the average barnyard has been included in the make-up of this drug. Here is abundant evidence of much needed improvement and it can best be accomplished by the introduction of this plant into cultivation and its subsequent improvement by breeding and selection. The testing of other species of Hyoscyamus may lead to the discovery of a more valuable form, and while Schneider says that the annual form of henbane is not desired medicinally, let us not despair since we find that this annual form has really never been tested thoroughly for its alkaloidal and therapeutic value.

Belladonna is in the same class with henbane, and while the range of species is not so large, still there is the variety lutea, which has so far never been tested. Seeds of this variety were brought to this country by Professor Henry Kraemer and through his kindness we now have this form growing in our experimental grounds. It offers an opportunity for hybridization with the official species Atropa belladonna. Sievers, of the Bureau of Plant Industry, has already shown what may be expected from consistent selection with the official belladonna. Further work of this nature, but leading to increased yield, together with a better understanding of soil and climatic condition is highly desirable. A similar series of investigations is suggested by the genus Datura. Here, however, the range of species is much greater and the variation in the nature of the alkaloids in the various species is such as to present problems of a different character, and which should lead to the use of some of these species for different specific purposes. The species Datura tatula has recently been shown to be equal to or better than Datura stramonium in the percentage of alkaloids. Both of these species have yielded to selection and hybridization in such a manner as to promise the early development of a much-improved strain. Turning to digitalis, we find a host of species and horticultural varieties of unknown therapeutic value which should furnish an abundance of material for the investigator. Many of these have been found to be extremely toxic, both in the flowering and nonflowering stages.

And so the discussion might be continued in detail for cannabis, conium, colchicum and any others that might be grown within the United States. Sufficient has been said, however, to draw attention to the many difficulties that may be encountered in attempting to grow medicinal plants upon a commercial scale, and of the opportunity and desirability for impovement. The beginner in this work should be advised to commence upon a small, experimental scale with a few of the most valuable drug-producing plants and enlarge his operations as results might indicate. The value of the work cannot be questioned, and while it is vitally important that medicinal plants be grown with the same care and consideration as in the case of corn, wheat or other economic forms, still the step from the wild plant to the highly developed variety of known constitution is a long one and cannot be accomplished in a few days.

It is hoped that nothing has been said which will tend to discourage the present widespread movement in drug cultivation. Rather, we would wish to encourage it but in such a manner as to lead to a fair understanding of the true state of affairs and to dispel the idea that large profits may be expected.

BOTANICAL DEPARTMENT,

# A CRITICISM OF THE PHARMACOPOEIAL DESCRIPTIONS OF VEGETABLE DRUGS

C. J. ZUFALL, B. S.

[A thesis presented to the faculty of Purdue University and reprinted from the Journal of the American Pharmaceutical Association, April 1915, p. 530.]

The inspection of crude drugs in a pharmaceutical manufactory requires continual reference to the descriptions of official drugs in the United States Pharmacopoeia. While these descriptions are considered authentic, the question often arises, are they really accurate and correct for commercial drugs? Comments and criticisms are repeatedly found in the pharmaceutical journals which leads one to suspect inaccuracy on the part of the pharmacopoeia committee which wrote these, and the purpose of this investigation is to determine how well these descriptions can be applied to the drugs of commerce and to authentic specimens.

The drugs were taken, one at a time, and large quantities of the material studied. The conditions for this study at the Eli Lilly and Company's plant are almost ideal, for more than a mere sample is seen, the entire contents of the bags and bales being examined as they are emptied in the mill-room for grinding. The pharmacopoeial descriptions were compared with as many authentic specimens as possible. The herbarium specimen was first checked up with the botanic authorities and then the crude drug, the herbarium specimen and the pharmacopoeial description compared. In many cases, three to five mounted specimens of the one species from widely separated regions were used, thus giving any variations due to climatic conditions.

## ACONITE

The six lots and ten samples examined show that the pharmacopoeial description is correct for Aconitum napellus except as to the thickness of the root. Many roots were found to be 35 mm. in diameter at the crown. The pharmacopoeia gives this diameter as "10 to 20 mm." It should be given as "10 to 35 mm. in diameter at the crown."

In order to aid in distinguishing the official drug from A. fischerii, which is being offered on the market as the true aconite, the pharmacopoeia should add that the "starch grains are 4 to 12 microns in diameter." Those of A. fischerii are much larger, being 10 to 22 microns in diameter.

## APOCYNUM

The pharmacopoeial description of Apocynum is: "The dried rhizome of Apocynum Cannabinum, *Linne*, or of closely allied species of Apocynum."

The phrase "or of closely allied species of Apocynum" should be omitted for this includes A. androsaemifolium, a "closely allied" species, so closely resembling A. cannabinum that it is difficult to distinguish between the two species, even when the plants are in flower. Yet A. androsaemifolium has different medicinal properties and is used under the name of "Bitter Root." The foremost pharmaceutical botanists admit that they do not know the difference and Dr. Henry Kraemer of the Philadelphia College of Pharmacy states that the only difference between these two roots is the presence of stone cells in the cortex of A. androsaemifolium and not in A. cannabinum. We have collected several specimens of the different species of Apocynum and are trying to establish the differences between these two species.

## BELLADONNA LEAVES

Belladonna leaves are often adulterated with the leaves and fruit of Scopola carniolica. The leaves of this adulterant resemble those of Belladonna so closely that it is almost impossible to detect it unless the fruit is present. So it seems wise to replace the phrase "frequently with the flowering tops intermixed," with the following, "usually with the tops possessing the dark brown or black, globular, indehiscent fruit, showing above the reflexed calyx lobes." This will exclude the tops of Scopola which usually possess the yellow or light brown, subglobular, transversely dehiscent fruit which is almost completely enclosed by the thin, paper-like calyx.

The requirement of 0.3 per cent. alkaloids is too low for belladonna leaves. In 61 assays of samples and lots made in the last five years only 3 have been as low as 0.3 per cent. The average of these 61 assays is 0.44 per cent. This indicates that the pharmacopoeial requirement is too low for prime drug and should be raised to at least 0.4 per cent.

## BERBERIS

During the examination of six lots, many Berberis roots were found to be 40 mm. in diameter so that the size should be changed from "3 to 20 mm." to "3 to 40 mm."

A peculiarity of Berberis not seen in other roots is that it splits upon drying. It would be well to state this in the pharmacopoeia as one of the characteristics of Berberis.

## BUCHU

It is impracticable to obtain Buchu without some stems. Some of these stems are thick, woody and undoubtedly inert, while "others are thin and contain the active constituent in considerable amount." (1)

The per cent and character of these stems should be controlled by the pharmacopoeia.

The stems in five lots and seven good samples offered for sale averaged 8 per cent. The pharmacopoeia should exclude Buchu containing more than 10 per cent. stems. The stems permitted should not be more than 1 mm. in diameter.

## CAPSICUM

During the last year it was found difficult to obtain the real Capsicum fastigiatum. The Japanese pepper was offered continually for Capsicum. The small Japanese variety answers the pharmacopoeial description but they are of a lower quality and are less pungent. The description should include the following and thereby exclude all but the genuine Capsicum: "The cells of the pericarp are usually quadrilateral, 20 to 55 microns in diameter, more or less wavy in outline, walls indistinctly beaded and (what is most noticeable) arranged in distinct longitudinal rows." (2)

## CARDAMOM

While the pharmacopoeia describes the cardamom "fruit" as official, it adds that "the seeds alone contain active and valuable constituents."

The seeds alone should be made official since they alone are of value and thereby lessen the amount of inert material in the preparations.

The inert pods may vary considerably, for some pods may be full while others may contain only a few seeds or may be empty. These empty pods being lighter than the seeds, will accumulate at the top of the bag on being handled. Thus the druggist may get a large per cent of pods in his first preparations made from a bag while there may be very few in the last preparations; thus his products will vary considerably.

Since the seed or "decorticated cardamom" is plentiful on the market, there seems to be no reason why it should not be made official instead of the "fruit."

## COCA

The requirement of "0.5 per cent. ether-soluble alkaloids" is too low.

The average of 21 assays of lots and samples is .85 per cent. ether-soluble alkaloids, the lowest assaying .66 per cent.

From these results it seems that the requirement should be raised to at least .75 per cent. ether-soluble alkaloids, for, if Coca leaves are properly gathered, properly shipped and properly stored, they will give a much higher assay than the pharmacopoeia requires.

## COLCHICUM SEED

The pharmacopoeia requires only 0.45 per cent. Colchicine which would include rather poor seed. The average of 18 assays is .66 per cent colchicine, the lowest being .53 per cent.

These results indicate that first class Colchicum seed will assay at least 0.6 per cent. colchicine and the pharmacopoeial requirement should be raised to this per cent.

## CUBEB

Cubeb berries grow in spikes and in gathering the drug the whole spike is picked, which includes the peduncle. In drying and handling the drug, the berries become detached from the peduncles which then occur as "stems."

Some berries on the market have no stems at all while others have an excess of them. This indicates that the stems are removed from a part of the drug, for which a higher price is obtained, and these stems mixed with other lots and the average price obtained for it.

If stems are not deliberately added to Cubebs they will make a rather constant per cent of the drug so the pharmacopoeia should specify that there shall not be more than 10 per cent. stems and these should not be more than two mm. in diameter.

## ERGOT

The examination of six lots consisting of 154 bags indicates that a few changes should be made regarding the color of the fracture and the pharmacopoeia made to read as follows: "fracture short, gray, pinkish, blue or purple," instead of "pinkish, or reddish white."

The thickness should be "from 3 to 5 mm." instead of "about 3 mm."

## GRINDELIA

The pharmacopoeia states "the dried leaves and flowering tops of Grindelia robusta and G. squarrosa" but does not state how much of the "tops". Since the activity of this drug evidently resides in the resinous exudation and since the resin is not found in the larger stems, the length of the "flowering tops" should be given so as to exclude the larger, inert stems. The maximum length should be 30 cm.

From the description in the pharmacopoeia, one would infer that the two species, G. robusta and G. squarrosa, were very much alike, whereas the leaves and scales of the involucres are distinct in the two species. The pharmacopoeia describes the leaves as "about 5 cm. or less long" whereas leaves 7 cm. long were found. "Lanceolate" should not be used in describing the leaves because that includes the leaves of G. Lanceolata while the leaves of the two official species do not approach the lanceolate shape. Instead of "pale green" the leaves vary from pale green to greenish yellow.

The leaves are further described as "somewhat coriaceous, and brittle". The leaves of Grindelia become very brittle upon drying and are not coriaceous.

From these discrepancies it seems best that the two species should be described separately because very often lots of drug arrive which are made up entirely of one species.

It seems that the following descriptions would be more scientific and workable than the one given in the pharmacopoeia:

Grindelia robusta; leaves 7 cm. or less long, broadly spatulate or or obovate, sessile, more or less clasping at the base, finely to coarsely serrate, obtuse, light green to greenish yellow, finely dotted, brittle; heads resinous-viscid, many flowered, depressed-urceolate, 1 cm. broad, composed of numerous imbricated, linear-lanceolate spreading bracts; ray-flowers yellow, ligulate, pistillate, sometimes absent; disk-flowers yellow, tubular, perfect; pappus of two or three mostly unequal awns about the length of the disk-flowers; taste pungent and bitter; odor aromatic and balsamic.

Grindelia squarrosa; similar to G. robusta except the leaves, which are 4 1-2 cm. or less in length and 8 to 15 mm. wide, usually decidedly oblong or oblong spatulate, obtuse, more or less clasping at the base, sharply spinulose-dentate, sometimes laciniate. The bracts of the involucre strongly squarrose.

## LUPULIN

The results of examining five lots and 16 samples indicate that prime Lupulin has an amber-yellow color which should be given instead of "brownish-yellow." The latter color is seen only in drug which is not fresh.

There have been suggestions that the maximum ash limit is too low, but from our experience we know that it is not. Careless handling or willful adulteration are the only causes for the presence of the foreign mineral substance which causes the high ash in some Lupulin.

## SAVIN

The pharmaceutical description of Sabina is not specific enough to exclude several other species of Juniperus; consequently, these other species are found on the market offered for savin. The oils of these species are inactive and undoubtedly this is the cause of savin falling into disuse.

It is difficult to identify a species of the Juniperus from a twig alone, without specimens of the fruit and without knowledge of the form and habit of growth of the plant. The difficulty is twofold. In the first place plants which undoubtedly belong to one species develop, if grown under varying conditions of soil and climate, very marked differences, both in general habit and in the character and mode of arrangement of the leaves. Juniperus sabina when found in the natural state, has decussate, ovate-lanceolate leaves, while the cultivated plant bears leaves which are more needle-like and sometimes in whorls of threes.

In the second place, plants of different species have adopted a similar habit; for instance, forms of J. sabina, J. phoenicea, J. thurifera, J. barbadensis, J. virginiana, L, and J. scopulorum, Sarg. possess closely imbricated, acute or acuminate leaves, tightly appressed to the stems, and are externally almost indistinguishable one from another.

So variable are some of the plants that, of J. Sabina, seven varieties are recognized by Biessner and described by him in his "Handbuch der Nadelholzkunde."

In 1902 there was published by the Ecole Supericure de Pharmacie, Paris, a thesis by H. Henri Mongin, entitled "Etude Anatomique de la fenille des Juniperinees." He pointed out that, in the drug of commerce, besides J. sabina, there were to be met with J. thurifera, L., and its variety gallica, and J. phoenicea, L. Most people would find it practically impossible to distinguish nonfruiting twigs of these species from the typical form of J. sabina. All have small leaves, closely appressed to the stem, giving the branches a cylindrical appearance. With fruiting specimens available, J. phoenicea is readily separated, having larger yellowish fruits, while the fruits of J. sabina and J. thurifera are small and bluish. resembling those of J. communis.

In 1874 M. E. Collin pointed out that the leafy twigs of J. phoenicea were substituted for those of savin in French Commerce. In 1902 M. Mongin found that they were still so employed in France, and indeed constituted the greater part of the drug met with in that country. He also points out that the oil of J. phoenicea is inactive, being similar to that of J. communis.

Mongin's work renders it possible to distinguish between J. sabina and these substitutes, even when only the young branchlets usually met with in the commercial drug, are available for examination. J. sabina and J. thurifera and its varieties have the leaves in pairs, each pair being at right angles to the succeeding pair, i. e. they are decussate. In J. phoenicea, on the other hand, the leaves are not decussate, but are arranged more or less spirally with five leaves inserted almost at the same level.

Mongin further shows that large sclerotic or stone cells are found in the mesophyll of the leaf of J. phoenicea and less abundantly in J. thurifera, whilst they are completely absent in J, sabina. Another point of difference is the continuance of the hypodermal layer over the resin duct in the leaf of J. phoenicea while in J. sabina the resin duct abuts directly on to the epidermis.

In 1905 Freeman stated that there occurred in commerce in Great Britain a form of savin "with more convex, less acute leaves, which are so closely appressed to the stem as to give a cylindrical appearance to the branchlets, very different it is true from the typical savin, but so closely resembling some of the forms of J. sabina that in the absence of fruit it is impossible to separate them by a microscopic examination." (3)

From these references we may conclude that savin has been adulterated for at least 40 years.

For some time there have been large quantities of so-called savin on the market which consists of twigs resembling somewhat the twigs of J. sabina, but the leaves are more convex, less acute and have an oval gland. By careful inspection it is noted that the arrangement of the leaves is not the same on all twigs. The drug is a mixture of about one-half twigs whose leaves are in whorls of threes and one-half twigs having decussate leaves. Branching twigs showed a peculiarity in that the branch had decussate leaves while the leaves of the twig were in whorls of threes. The latter fact indicates that the pharmacopoeia is at fault for these branchlets answer its requirements while the main twig of the same species does not.

In microscopic sections of this substitute, both the decussate leaves and those in whorls of threes, show the following characteristics: large sclerotic or stone cells in the mesophyll beside the resin ducts, and the hypodermal layer extending between the resin ducts and the epidermis.

A pressed herbarium specimen of J. Sabina showed the leaves all decussate, apex decidedly acute, longer, less convex and the gland linear-oblong. Microscopic sections show the absence of sclerotic or stone cells and the resin duct abuts directly on to the epidermis. These characteristics indicate that according to M. Mongin, the pressed specimen is properly named and that its substitute is not obtained from J. sabina. The leaves of the commercial drug are not arranged spirally hence it is not obtained from J. phoenicea and the leaves are not all decussate, hence it is not J. thurifera. But pressed specimens of J. occidentalis, Hook, have short, oval, convex leaves with stone cells beside the resin duct and the resin duct separated from the epidermis by the hypodermis. This form shows the peculiarity of having the leaves in whorls of threes on the large branches but the branchlets from these have decussate leaves. This indicates that the substitute which has been offered for savin is undoubtedly from J. occidentalis.

The specimens ot J. sabina obtained from the New York Botanic Garden show the following characteristics in contrast with those of this substitute: leaves spreading, acuminate, all decussate, no stone cells in the mesophyll and the resin duct abutting directly on to the epidermis.

In order to exclude all but J. sabina the pharmacopoeial description should read as follows:

Thin quadrangular branches bearing green, decussate leaves which are scale-like, ovate-lanceolate, acute or acuminate, appressed or spreading, imbricated; having on the back a shallow groove containing a linear-oblong gland; odor strong, penetrating, terebinthinate; taste disagreeable, resinous and bitter. In cross-section the leaves should show no stone cells and the resin duct abutting directly on to the epidermis."

The fact that the pharmacopoeia states that the leaves are dark green and have an oblong or roundish gland indicates that the description may have been made from commercial drug and not from known specimens of J. sabina.

## SCOPARIUS

A more definite description would be otained if "thin flexible branched twigs 2 to 3 mm. thick, externally dark green" were replaced by "flexible, round, light green branches with five low narrow ridges, 2 to 3 mm. thick; the smallest branches being uniformly 1 to 1.5 mm. thick, dark green, with five distinct wings."

## VIBURNUM OPULUS

The pharmacopoeia states: "The dried bark of Viburnum opulus Linne", and then describes the bark of Acer spicatum. Consequently the Cramp Bark on the market for the last twenty years has been the bark of Acer spicatum and, although it answers the pharmacopoeial description, it is not the bark desired.

In 1912 Nathan S. Slatter, of Bennington, Vt., discovered that the Cramp Bark on the market was not the same as the bark of Viburnum opulus but was that of Acer spicatum. He exposed the fact to Oliver A. Farwell, of Detroit, who confirmed Slatter's statement by comparing Cramp Bark of commerce with authentic specimens of Viburnum opulus and Acer spicatum.

Farwell found that in 1895, a pharmaceutical committee of

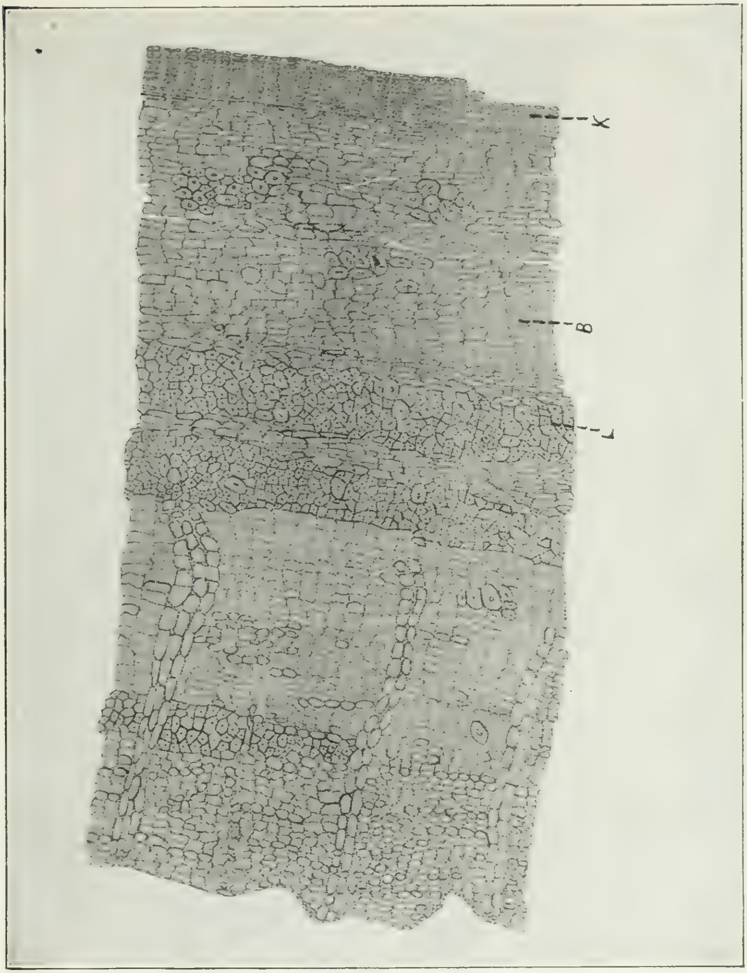

PLATE I—Cramp Bark, Commercial Drug. Transverse Section of Bark.
B. Bark; L. Bast; K. Corky Layer.

revision which tried to show the difference between Viburnum opulus and V. prunifolium, used Acer spicatum for an authentic specimen of Viburnum opulus. This specimen was obtained from a large pharmaceutical manufactory and indicates that A. spicatum was used at that time for Cramp Bark. (4)

The sixth revision of the pharmacopoeia under Viburnum opulus gives characteristics undoubtedly those of Acer spicatum. Such characteristics as "fracture tough, the tissues separating in layers" are surely not those of V. opulus, for the bark of the latter is very brittle and gives a sharp, clean, fracture.

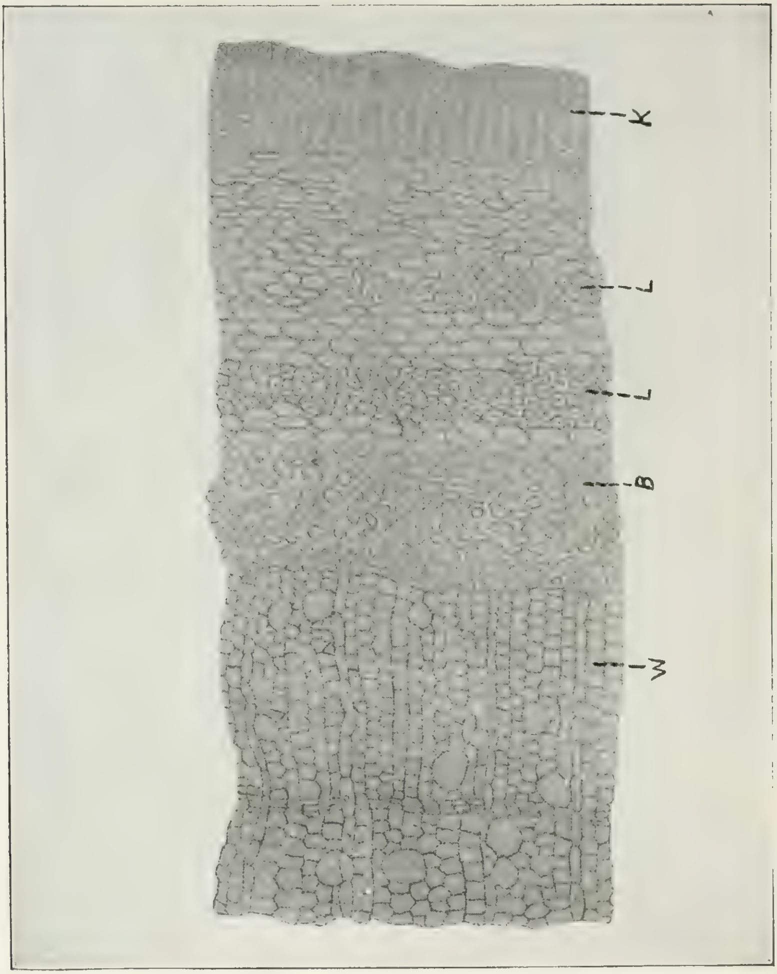

PLATE II—Acer Spicatum. Transverse Section of Bark and Wood.
B. Bark; L. Bast; K. Corky Layer; W. Wood.

The findings of these two men led F. A. Miller to investigate the situation. He obtained specimens of both Acer spicatum and Viburnum opulus from Arnold Arboretum, Missouri Botanic Garden, Biltmore Nursery, Kew Botanic Garden and the German Botanic Garden. Sections and drawings of these were made and of commercial Cramp Bark. See plates, I, II and III. These drawings show clearly that commercial Cramp Bark is taken from Acer spicatum for both show the bands of bast fibres and the absence of calcium oxalate.

The accompanying illustrations are representative of all the draw-

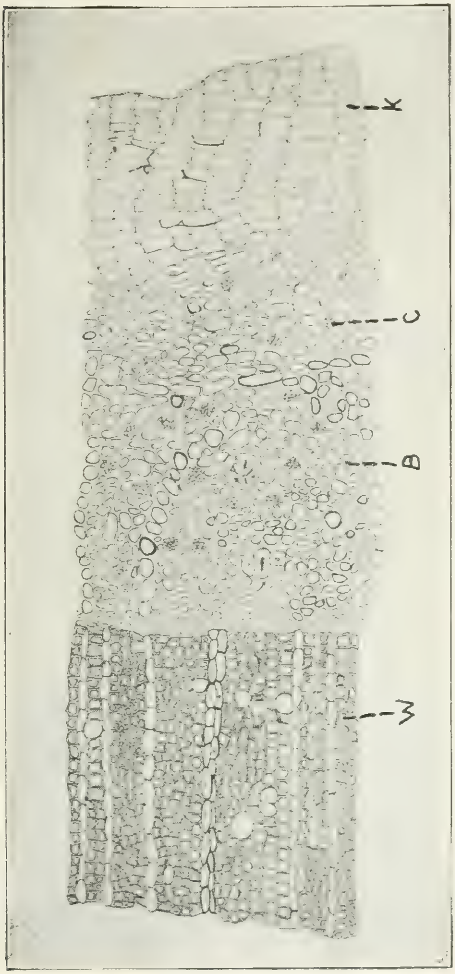

PLATE III—Viburnum Opulus. Transverse Section of Bark and Wood. B. Bark; W. Wood; C. Calcium Oxalate Crystals; K. Corky Layer.

ings made and show the desired points.

The pharmacopoeia should replace the present description by the following: "In transversely curved pieces or quills, of variable length, and one mm. or less thick; outer surface gray, longitudinally furrowed, small oval lenticels; inner surface brownish yellow, very smooth; fracture short and clean; transverse sections show an abundance of calcium oxalate crystals in rosettes, no bast fibres; taste at first bitter and then astringent."

## VIBURNUM PRUNIFOLIUM

Two characteristics of this drug should be added which will aid considerably in its identification. These are the sour odor and the peculiar short, smooth striae on the inner surface.

The following is a list of the drugs and the number of shipments inspected, whose pharmacopoeial descriptions are correct as far as could be determined:

| | | | |
|---|---|---|---|
| Arnica | 17 | shipments | inspected |
| Aurantii amari cortex | 5 | " | " |
| Aurantii dulcis cortex | 4 | " | " |
| Belladonna radix | 9 | " | " |
| Calenduia | 5 | " | " |
| Calumba | 6 | " | " |
| Carum | 3 | " | " |
| Caryophyllus | 7 | " | " |
| Chirata | 6 | " | " |
| Cimicifuga | 12 | " | " |
| Cinchona | 11 | " | " |
| Cinchona, Red | 7 | " | " |
| Cinnamon Ceylon | 4 | " | " |
| Cinnamon Saigon | 9 | " | " |
| Colchicum Corm | 5 | " | " |
| Colchicum Seed | 3 | " | " |
| Colocynth | 14 | " | " |
| Conium | 3 | " | " |
| Convallaria | 4 | " | " |
| Cotton Root Bark | 11 | " | " |
| Cypripedium | 3 | " | " |
| Digitalis | 9 | " | " |
| Eriodictyon | 6 | " | " |
| Eucalyptus | 5 | " | " |
| Euonymus | 21 | " | " |
| Eupatorium | 2 | " | " |
| Fennel | 5 | " | " |
| Frangula | 8 | " | " |
| Gelsemium | 6 | " | " |
| Gentian | 12 | " | " |
| Hamamelis Leaves | 6 | " | " |
| Hydrastis | 28 | " | " |
| Jalap | 9 | " | " |
| Kousso | 3 | " | " |
| Lappa | 14 | " | " |
| Leptandra | 8 | " | " |
| Licorice Root | 18 | " | " |
| Lemon Peel | 10 | " | " |
| Lobelia | 5 | " | " |
| Matico | 5 | " | " |

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*Botanical Department.*

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# CONCERNING THE U. S. P. STANDARD FOR PITUITARY EXTRACT (LIQUOR HYPOPHYSIS).

C. R. ECKLER, Ph. C., M. S.

[Reprinted from the American Journal of Pharmacy, Vol. 89, page 195, May, 1917]

The United States Pharmacopoeia places a physiological standard requirement for pituitary extract (Liquor Hypophysis) which reads as follows:

"One mil of Solution of Hypophysis, diluted 20,000 times, has the same activity on the isolated uterus of the virgin guinea pig as a 1:20,000,000 solution of beta-imidazolylethylamine hydrochloride when tested as directed by the United States Hygienic Laboratory."

Attention has been called to the fact that this is an exceedingly low requirement, but the correctness of the figures in the statement has not been questioned. The results of my work on the subject are so utterly at variance with the pharmacopoeial statement that I am prompted to question whether or not the requirement as stated is correct. My question is this: should the equivalent of 1:20,000,000 B-imid. be 1:20,000 or 1:200,000? In order to show my grounds for raising this question, I wish to briefly describe my apparatus (a detailed description of which appears in another paper), to give in detail the method and procedure of making the dilutions, together with my results in general as to the relative activity of the two substances in causing contractions of the isolated guinea pig uterus.

## APPARATUS.

The apparatus used is similar to, and was fashioned after, that described by Dale and Laidlaw (1), and is in principle essentially like that used by Roth (2). It consists of an amber-glass, 100 Cc., graduated chamber for holding Locke's solution in which the uterus is immersed, surrounded by a water bath kept warm by an immersed carbon lamp painted black. From an elevated container, Locke's solution is passed down through a coil in the water bath (thereby warming it as needed) and up into the amber glass chamber, filling the chamber from the bottom, at which place there is also an out-let. Warmed air (warmed by passing through another coil in the bath) is made to gently bubble through the Locke's solution, thereby keeping it aerated and circulating. The water in the outer bath is also kept circulating by a current of air in order to maintain an even temperature throughout the bath. The amber glass chamber bears a cork stopper at the top carrying a thermometer and the tube conveying the air, to which the uterus is attached. The stopper has two holes, one for the thread to the writing lever, and one through which to inject the pituitary solution. In this all glass apparatus light is practically excluded from the uterus, except for that which passes through the amber glass, and at the same time all the contents may be readily observed, rendering easier the control of dilutions, temperature, emptying of the chamber, rate of air flow, and so on.

## MATERIAL.

The uteri of virgin guinea pigs weighing from 175 to 250 grams have been commonly used. Larger pigs, up to 300 grams have usually proved less satisfactory.

## METHOD AND PROCEDURE.

The animal is killed by instantaneous decapitation. The whole uterus, with a small section of vagina but without ovaries, is removed and placed in a fold of cotton saturated with warm Locke's solution until a horn can be separated, attached to the tube, and placed in the chamber. The vaginal end of the horn is tied to a little post on the air tube by means of a silk thread, which is sewn into the peritoneal covering on the side of the broad ligament. The ovarian end of the horn is attached to the thread running to the writing lever by a small pin hook which is passed through the Fallopian tube, or, if the horn is unusually long, a part of the ovarian end is removed and the hook is passed through the peritoneal fold as before. The tube bearing the uterus is then placed in the chamber, the thread attached to the writing lever, the uterus weighted down with from 1 to 4 grams (usually 1½ to 3), the writing lever brought to the drum and the air tubes connected, the temperature having been previously adjusted to between 37.5 and 39.5° C.

After spontaneous movements have appeared, usually after about half an hour, the application of pituitary extract dilutions are begun and are repeated every 20 to 30 minutes depending upon the time required for relaxation by the given organ, approximately the same period of application being maintained for each uterus throughout the experiment. A dilution of the pituitary solution is sought which will produce contractions equal to those recorded under the standard.

## MANNER OF MAKING DILUTIONS.

It should be understood that when "pituitary extract" is mentioned in this paper, the commercial solution is referred to. The dilutions are made as follows: .1 c.c. of the pituitary extract or solution is drawn from the ampoule by means of a narrow pipette and diluted with distilled water to 100 c.c. in a volumetric flask. This makes a 1:1,000 dilution. I use distilled water in this place believing that the dilution so made will not lose strength materially during the experiment of 5 or 6 hours. The deterioration of pituitary extract in Locke's solution has been mentioned by Fenger (3). When it is desired to apply say a 1:200,000 dilution to the uterus. .5 c.c. of this 1:1,000 dilution is drawn off and added to the 100 c.c. chamber. The Locke's solution in the chamber is kept at such a height that when the pituitary dilution is added, the 100 c.c. mark will have been approximately reached. If a trifle more Locke's solution is needed it is allowed to flow gently into the chamber from the reservoir. The dilutions of pituitary extract are measured out with a narrow pipette into a small, conical, glass cup and diluted with enough Locke's solution to make about 1 c.c. From this glass the dilution is taken up with a "Record" syringe having a long needle, and introduced into the chamber, the glass and syringe being afterward rinsed with another 1 c.c. of Locke's solution.

The beta-imidazolylethylamine hydrochloride was supplied by the Hoffmann La Roche Co. .05 gram of this substance taken from the 1 gram vials as supplied, without desiccating (being apparently dry), is placed in a sterile 500 c. c. volumetric flask and dissolved in freshly boiled and cooled distilled water containing a small amount of Acetoform and made up to the mark. This 1:10,000 dilution is sealed in ampoules and kept in a refrigerator in the dark. It seems to keep thus, for many months, without noticeable deterioration. For use, 10 c.c. are diluted to 100 c.c. in a volumetric flask. Of this 1:100,000 dilution, .2, .25, .4, or .5 c.c. are added to the 100

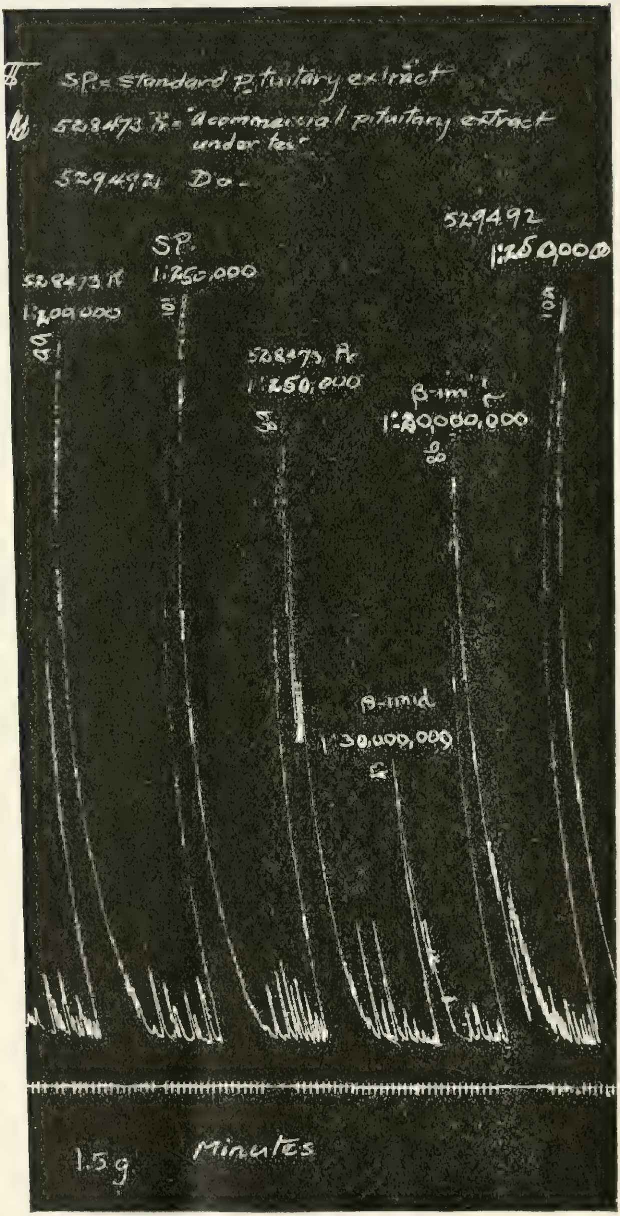


FIG. 1. Kymographic tracing comparing a standard pituitary extract with commercial extracts.

c.c. chamber containing the uterus when a dilution of 1:50,000,000, 1:40,000,000, 1:25,000,000, or 1:20,000,000 respectively is desired.

## COMPARATIVE ACTIVITY.

During the past three years I have made over 500 kymographic tracings of the isolated uterus in testing pituitary

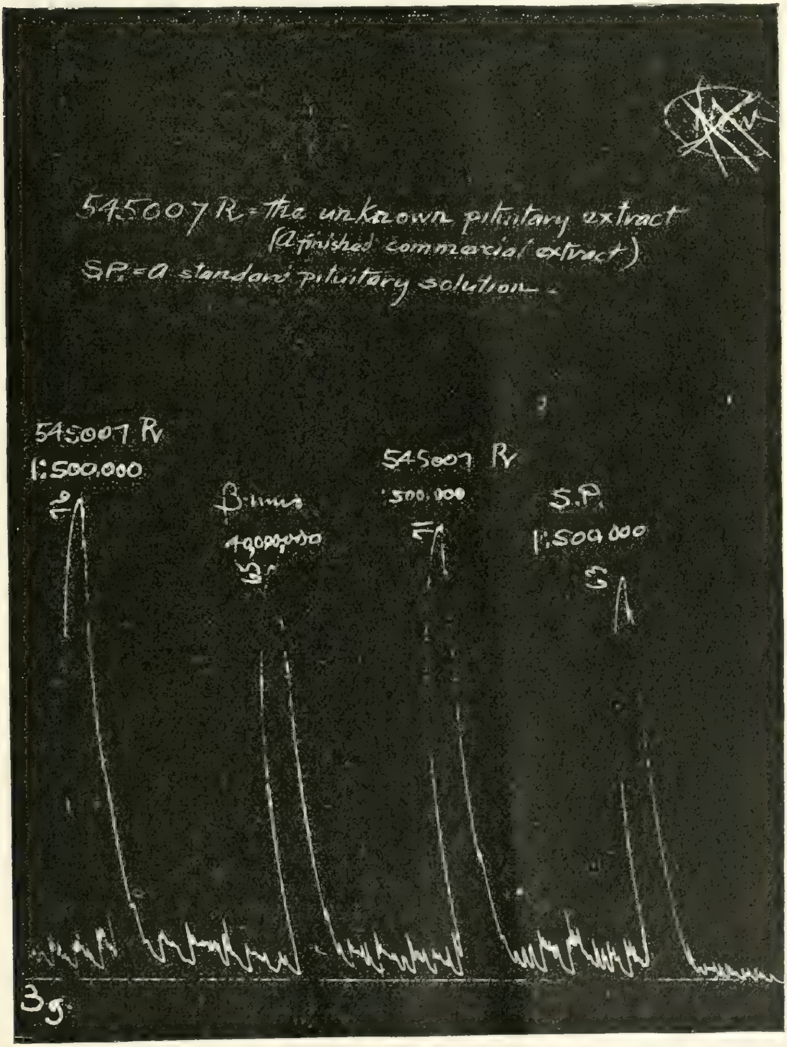


FIG. 2. Kymographic tracing comparing a standard pituitary extract with a commercial extract.

glands, solutions, and finished commercial extracts made in this laboratory and also in others, and during this experience I have never found it necessary to apply as low a dilution as 1:20,000 of any commercial extract within the age limit. Indeed, it is rare that I ever apply a 1:100,000 dilution. The uteri on the average have required dilutions of from 1:160,000 to 1:600,000 occasionally 1:800,000 to 1:1,000,000 and rarely 1:2,000,000. This fact by itself is not of much importance since some uteri are extremely sensitive, but it is significant in connection with the fact that my dilutions of B-imid. effective on these same uteri have been about that directed in the pharmacopoeia, that it, usually from 1:16,000,000 to 1:40,000,000. Occasionally a greater dilution of B-imid. has been necessary but rarely a lower. My experience with first-class pituitary extracts has led me to adopt as a standard requirement, that a 1:250,000 dilution should equal 1:20,000,000 B-imid. (This dilution of the extract has no reference to the amount of gland contained, but means 1 part of finished extract as drawn from the ampoule to 250,000 parts of Locke's solution.) In order to illustrate this relationship, a few self explanatory tracings are here reproduced. As evidence that this is about what one would expect of a carefully prepared pituitary extract, let us consider the following:

Fenger (3), in testing fresh posterior lobes from full grown cattle and calves, found the following dilutions of a 5 per cent. solution to be equivalent to 1:20,000,000 B-imid. solution:

| | |
|---|---|
| Full grown cattle | 1:38,000 |
| Calves | 1:40,000 |

Let us use the 1:40,000 dilution for convenience. Since 1 part of a 5 per cent. extract of fresh posterior lobe diluted 40,000 times is equivalent to 1:20,000,000 B-imid., then the fresh lobe itself would be equivalent to this standard in a dilution 20 times as great, or 1:800,000. Now if a commercial extract were made from such material, without loss of activity, so as to represent .2 to .3 grams of fresh lobe per 1 c.c. (which is about what commercial preparations represent) then we should expect such an extract to be equivalent to the standard in two-tenths to three-tenths as great a dilution, or 1:160,000 to 1:240,000.

Roth (4) gives a table in which he states that a 1:1,000,-

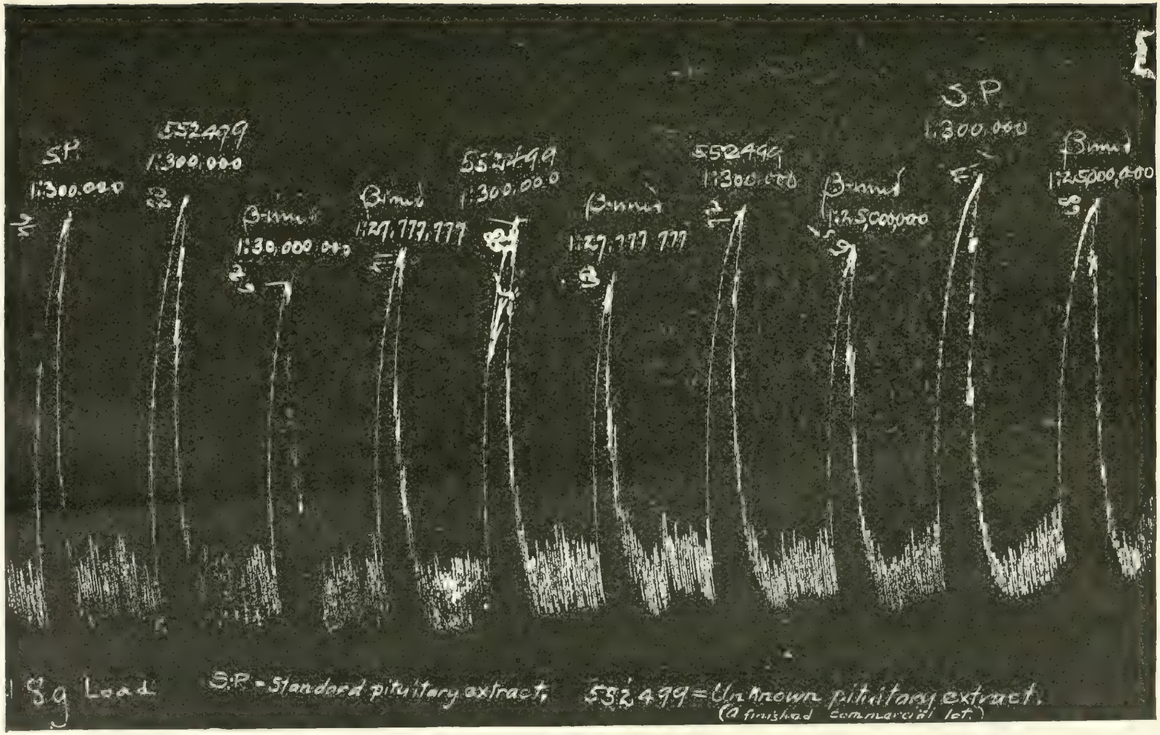


FIG. 3. Kymographic tracing comparing a standard pituitary extract with a commercial extract.

000 dilution of fresh posterior lobe from a steer is equivalent to 1:20,000,000 B-imid. (Although he says in his heading, "Concentration of extract," it is assumed that he actually means concentration of fresh posterior lobe since in a previous paragraph he states "the amounts mentioned in the following tables are in terms of fresh material.") Reasoning from these figures, if a commercial extract were made from such material as the steer's lobe here mentioned, without loss of activity, so as to represent .2 to .3 grams per 1 c.c., then we should expect such an extract to be equivalent to the standard in two-tenths to three-tenths as great a dilution, or 1:200,000 to 1:300,000. It may be noticed that these figures compare favorably with those I have obtained in testing first-class commercial extracts.

If finished commercial extracts are equivalent to the standard in dilutions of only 1:20,000 as stated in the U. S. P., what is the explanation of this great difference? Are manufacturers suffering a loss in activity amounting to 90 per cent. or more, due to their manufacturing processes? Why is it that extemporaneous preparations as made by Roth and Fenger will show 10 to 15 times as great activity as is shown by carefully made commercial preparations? Is this, in truth, the case, or is the standard as stated at fault? From my experience I would say that the pharmacopoeial standard for Liquor Hypophysis is about one-tenth that of average commercial pituitary extracts. These pituitary extracts have been on the market for several years and have been used by physicians everywhere, who have become familiar with their degree of activity and have become accustomed to giving certain doses. It seems advisable therefore, that instead of lowering the activity of pituitary extracts to meet the U. S. P. requirement, the U. S. P. requirement should be raised so as to compare favorably with these preparations which have become so well known.

Finally, I wish to express my obligation to Dr. A. L. Walters, under whose supervision this work has been carried out.

### *References.*

(1) Jr. Pharm. and Exp. Therapeutics, 1912-13, No. 4, p. 75.
(2) Hygienic Laboratory Bulletin No. 100.
(3) Jr. Biological Chem. 1916, Vol. 25, p. 417.
(4) Hygienic Laboratory Bulletin No. 109. (Table 9.)

*Department of Experimental Medicine.*

# APPARATUS FOR STUDYING THE EFFECT OF DRUGS ON THE ISOLATED GUINEA PIG UTERUS.

C. R. ECKLER, Ph. C., M. S.

[Reprinted from the Journal of Laboratory and Clinical Medicine, Vol. II, p. 819, August, 1917].

Those who have occasion to study the effect of drugs on isolated organs suspended in Ringer's or Locke's solution, such as strips of intestine or the various structures from the male and female genital tracts, and particularly those who are engaged in the assay of ergot and pituitary products on the isolated guinea pig uterus, may be interested in a form of apparatus, here described, which has proved very convenient and useful for such purposes.

This apparatus was fashioned somewhat after that described by Dale and Laidlaw (1), and in its original form, was first set up and used in this laboratory by W. F. Baker. It differs from similar apparatus chiefly in being practically all glass. During the three years or more in which the apparatus has been in almost constant use, several changes and additions have been made that seem to be decided improvements.

In its present form (Figure A), the apparatus consists of two units, each unit being complete by itself for the carrying out of an experiment. With the two units an assay of pituitary extract, for example, can be carried out in duplicate, or, two samples can be compared with a standard, one sample with the standard in one unit, and the other sample with the standard in the other unit. And with this duplicate form of apparatus both horns of a uterus may be utilized, one in one unit and one in the other.

The essential part of each unit is a water bath with included coils, tubes, etc. The water bath (1, Fig. B) consists of a bell glass which is inverted and set in a large ring on a heavy ring stand. This bell glass measures 15 Cm. wide by 20 Cm. deep, inside, and has a capacity of about three liters after the other parts are in place. In its inverted position, the bell glass has one tubulature near the top (3), and one at the bottom (2). The top or large opening of the bell glass is covered with a disc of sheet metal (German silver) (4), through which pass the various tubes, etc. The tubulatures are closed by rubber stoppers (5 and 5-a). The bath is filled with distilled water to a height regulated by an overflow tube (6). Another tube (6-a), connected with the overflow tube, allows the bath to be drained.

The water in the bell glass is kept warm by means of a tubular form carbon lamp (7). The size of lamp needed will depend on the room temperature, but 30 Watt lamps have been used almost entirely. The lamp can be immersed full length in the bath or only part way, as necessary. When about the right depth is found, the room temperature remaining constant, very little attention is required to keep the temperature of the bath, indicated by the thermometer 32, practically constant. In order to maintain an even temperature throughout the bath, the water is kept continually circulating by gently passing through it a current of air which is conveyed to the bottom of the bath by a glass tube (8). In order to exclude the light of the lamp from the uterus or other material used, the lamp is painted black.

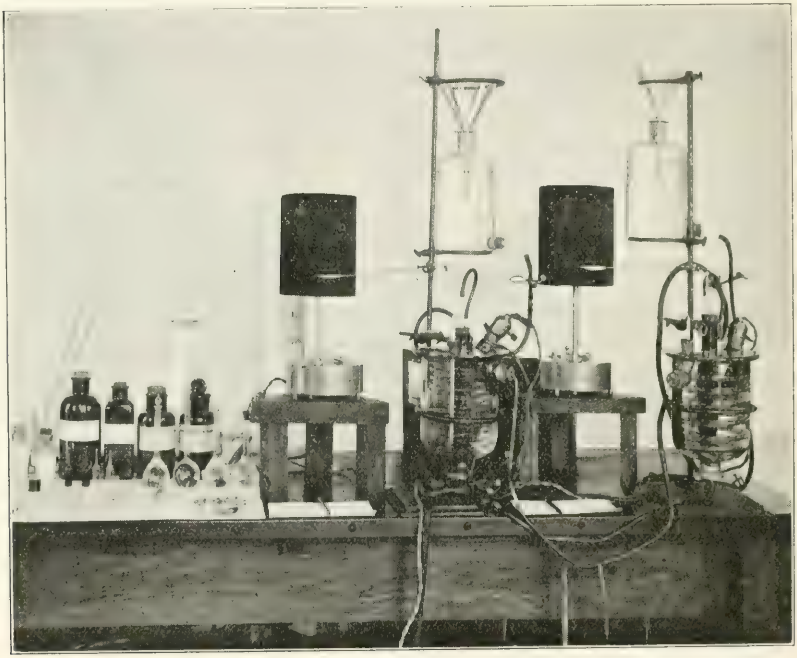


Fig. A.

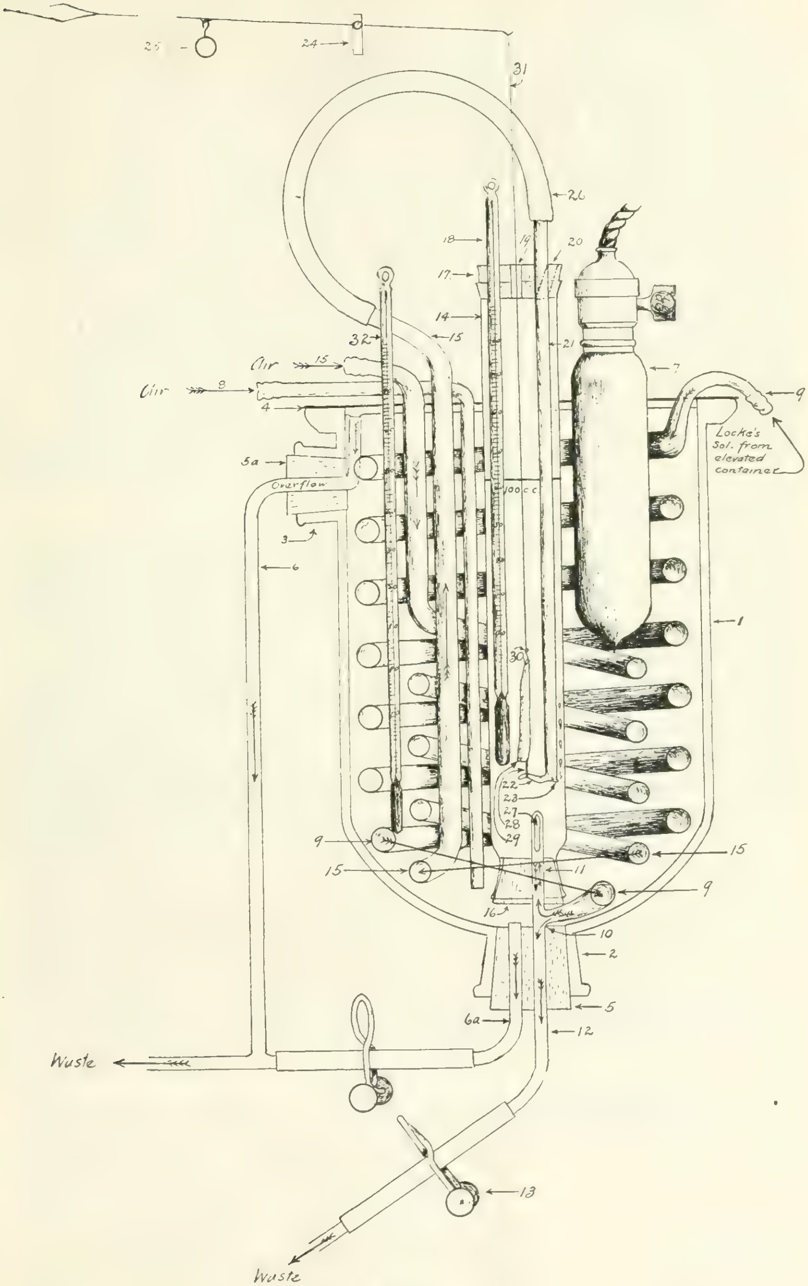


FIG. B.

View of apparatus, partly in section.

Two glass coils are set in the bath, the outer one (9) having a capacity of 200 Cc., is for the purpose of warming the Locke's solution which is allowed to flow into it from an elevated container—a 2 liter aspirating bottle. By this method of warming the Locke's solution in small quantities only as needed, precipitation of calcium salt is avoided which would to some extent occur if the whole container of solution were kept warm for some time. The lower end of this coil is joined to a tube forming a T (10), one arm of which (11) passes up through the lower stopper of an amber chamber (14), and the other arm (12) passes down through the stopper at the bottom of the bell glass. By means of a pinchcock at the elevated container (Figure A) and the pinchcock 13, the Locke's solution may be flowed down and through the coil and either up into the chamber by way of tube 11, or down to a waste bucket by way of tube 12, and, of course, the chamber may be emptied by way of tubes 11 and 12.

The inner coil of the bath (15) is for the purpose of warming the air or oxygen which is to bubble through the Locke's solution in the chamber. The main object of warming the air is to reduce the stimulation which the cool air would give to the uterus in passing about it when the chamber is momentarily emptied. Each time the chamber is emptied, one may stop the air from coming to the apparatus by pinching the rubber air tube, but this is somewhat inconvenient when two pinchcocks, in addition, have to be operated quickly. Managing any way one may, it seems that the emptying of the chamber will surely cause slight stimulation of the uterus, which may result in very slightly increased contractions. During the changing of the solution, however, one may stop the drum and if the writing lever or clamp holding it can be adjusted by a thumb screw, the writing point may be drawn away from the drum, and thus the record will not be disfigured by such added contractions.

The chamber (14) for holding the Locke's solution, in which the uterus or other organ is immersed, is made of amber glass tubing (3 Cm. inside diameter) and is graduated to 100 Cc. It bears a cork stopper at the top and a rubber one at the bottom. As previously mentioned, the chamber is filled through tube 11 from coil 9, and is emptied through tubes 11 and 12, the solution flowing off to a waste bucket or drain. Tube 11 has a blind end in the chamber but is slotted on two sides (27) to prevent the current of Locke's solution from striking directly against the uterus when the chamber is filled. The stopper at the top of the chamber holds a thermometer (18) and a tube conveying air or oxygen to the Locke's solution. This stopper also has two holes, one (19) for the thread (31) from the uterus to the writing lever, and another (20) through which the pituitary solution or other drug is introduced.

The introduction of the drug may conveniently be made with a "Record" syringe having a long needle by means of which the drug can be injected directly into the Locke's solution. The air tube (21) has a small post about 1.5 Cm. long near the bottom (22) to which the uterus (29) is attached, and the opposite side of the tube is drawn out into a little point or jet (23) from which the air escapes into the Locke's solution. The tube receives the air from coil 15, which in turn receives it from the tank or compressed air system of the building. Coil 15 is connected with tube 21 by a rubber tube (26).

The thread from the uterus is attached to one arm of a counterbalanced Harvard heart lever (24), the other arm being weighted down (1 to 4 Gm.) by hanging upon it small lead weights (25). Harvard slow speed, single kymographs are used. These instruments are made to turn the drum once in ten hours, but for this purpose are speeded up to about one revolution in eight hours by diminishing the weight of the pendulums.

For the purpose of giving a clear understanding of the operation and use of this apparatus, the testing of pituitary extract will be very briefly outlined. A somewhat more detailed description of the method is given in another paper (2).

## MATERIALS.

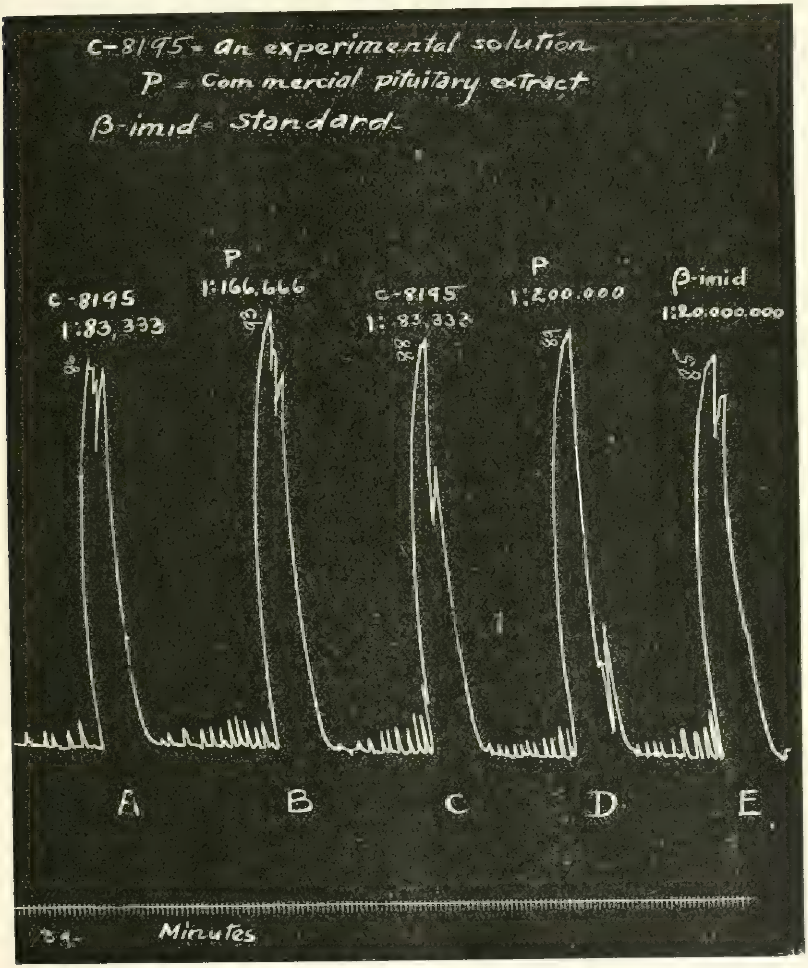
1. Chemically pure salts necessary for making Locke's solution.
2. Distilled water.
3. Pituitary extract to be tested.
4. B-imidazolylethylamine hydrochloride, or standard pituitary substance.
5. The uteri of 175 to 250 Gm. virgin guinea pigs.

## PROCEDURE.

The distilled water is warmed to 39° C. and introduced into the bell glass. The lamps are turned on and adjusted to the proper depth to maintain the desired temperature. While this adjusting is going on, the Locke's solution may be made up and introduced into the elevated chamber, the dilutions of the extract to be tested and the standard may be made, the drums smoked, the needles threaded, etc. When the apparatus is in readiness, the pig is decapitated, the entire uterus with Fallopian tubes and section of the vagina removed to a fold of cotton saturated with warmed Locke's solution and the horns of the uterus separated, excluding the section of vagina. The vaginal end of a horn is tied to the little post on the air tube (22) by a silk thread (28) which is sewed into the peritoneal covering on the side of the broad ligament. The ovarian end of the horn is attached to the thread (31) running to the writing lever by a small pin hook (30) which is passed either through the Fallopian tube or through the peritoneal covering as before.

The writing lever is weighted, and with a thumb-screw adjustment, the writing point is brought to the drum and the drum started. As soon as spontaneous movements of the uterus have appeared, which will usually be in about half an hour, the application of the standard substance is begun. It is first determined whether or not the uterus will react in a quantitative manner and, if so, the standard and unknown are then given alternately until a dilution of each is found which will produce like contractions of the uterus. For example, it may be found that a 1:200,000 dilution of the pituitary extract will cause approximately the same extent of contraction as a 1:20,000,000 dilution of B-imidazolylethylamine hydrochloride. (D and E on kymogram.)

The substances to be tested are carefully measured out with narrow pipettes and introduced into the 100 Cc. chamber with a long-needled "Record" syringe, in such strength of solution that the final dilution in the chamber will bring the substances to the dilution desired. For example, if it is desired to apply a 1:250,000 dilution of the pituitary extract to the uterus, 0.4 Cc. of a 1:1,000 solution may be introduced into the 100 Cc. chamber and the fluid, which will then measure about 99 Cc., made up to mark by rinsing the syringe and dose cup with sufficient warm Locke's solution, thereby diluting the 1:1,000 solution 250 times. Or, if it is desired to apply a 1:20,000,000 dilution of B-imid., 0.5 Cc. of a 1:100,000 solution may be introduced into the 100 Cc. chamber and the fluid made up to mark as before, thereby diluting the 1:100,000 solution 200 times. The bubbling of the air



through the Locke's solution equally distributes the injected solution in a few seconds.

After the application has been made and the writing point has registered the greatest contraction the uterus will make with the given dilution of drug, which will usually require from three to five minutes, the chamber is emptied by opening pinchcock 13 when the fluid will flow out through tubes 11 and 12. Closing this pinchcock and opening the one at the elevated container, Locke's solution will flow in from coil 9 and fill the chamber. In order to remove all considerable traces of the drug which may have adhered to the sides of the chamber or diffused from the wall of the uterus, the chamber is emptied again and refilled to within 1 or 2 Cc. of the 100 Cc. mark. After about 15 to 30 minutes, depending on the activity of the particular uterus, another application of drug is made, and so on, until the end result is reached.

### *References.*

(1) Jr. Pharm. and Exp. Therapeutics, 1912-13, p. 75.
(2) Am. Jr. Pharmacy, 1917, V. 89, No. 5.

*Department of Experimental Medicine.*

# SLIDE HOLDER AND PROTECTOR FOR THE C. S. & E. WARMING STAGE.

C. R. ECKLER, Ph, C., M.S.

[Reprinted from the Journal of Laboratory and Clinical Medicine, Vol. I, page 191].

One of the most widely useful accessories for the microscope in the field of biology is the warming stage and incubator. For observation of bacteria and yeasts in the hanging drop, for the study of malarial parasites, trypanosomes, amœbæ, or other motile organisms, for the carrying out of Widal tests, etc., such an instrument is not only of inestimable value but is almost indispensable.

A very convenient electrical warming stage and incubator is found in the No. 8, made by the Chicago Surgical and Electrical Company. As a stage incubator this instrument (Fig. 1) seems quite complete and for a description of it, quotation may be made from a booklet supplied by the Arthur H. Thomas Company, Philadelphia:

"The outside dimensions are 3½"x2"x½" in thickness. The incubating chamber measures 3"x1"x½" in depth and has a removable glass bottom consisting of a regular 3x1 slide. The specimen to be examined is placed on this slide. The condenser may thus be brought up close to the glass, same as under ordinary conditions, permitting the use of the oil immersion lens.

"To cover top of incubator we furnish a piece of clear mica having a hole cut in center through which the lens is passed when making adjustment for the bottom slide. A rubber washer slipped over the lens is provided for sealing the opening between the lens and mica.

"By this arrangement a perfect incubating chamber is obtained, where bacteria may be observed for any length of time.

"The incubator is complete in every detail with heating element, thermometer and adjustable electro-thermostat, which automatically maintains a constant temperature inside the incubating chamber, as long as the electric current is on.

This device can be adjusted for any temperature between 80° and 110° Fahrenheit and is very accurate.

"To operate, simply screw the current tap with its lamp into a 110 volt electric lamp socket and turn on the current."

This description appears to be correct except for the first paragraph in reference to the size of the incubating chamber (I. Fig. 1), which measures approximately 2⅜″x¾″x½″, instead of 3″x1″x½″, and carries a slide (G, Fig. 1) at the

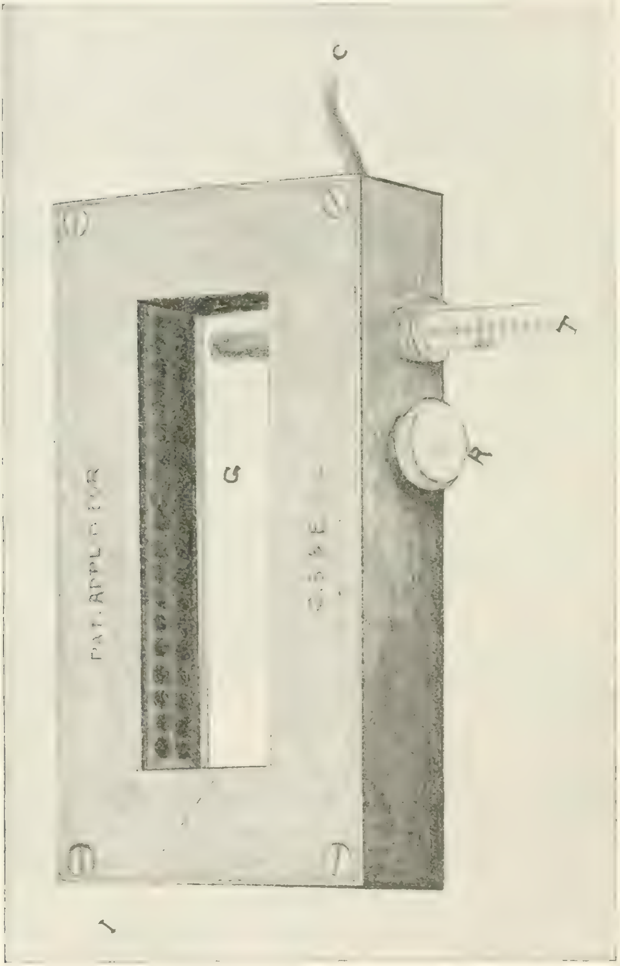


FIG. 1. Warming stage and incubator. Mica cover for incubating chamber not shown. C, cord from resistance lamp; T, Thermometer; R, Thumb-screw for regulating temperature; I, Incubating chamber; G, Glass slide at bottom of chamber.

bottom of the incubating chamber measuring approximately 2⅜″x¾″ instead of 3″x1″.

This instrument may be used as well for a warming stage, and perhaps in this way offers its greatest field of usefulness. For use in this manner it seems quite complete with one exception. Dr. A. L. Walters, using one of these instruments in the study of the endamoeba of pyorrhoea, suggested the need of a slide holder and protector, there being no device on the instrument to hold the slide firmly in position during the extended observation of the motile forms, or to protect the slide from cross currents of air, or to seal any small spaces between the edges of the slide and the top of the incubator. Such spaces were found, owing to the fact that the top of the incubator was not perfectly level at all points. To fill these needs, the attachment here described, was constructed.

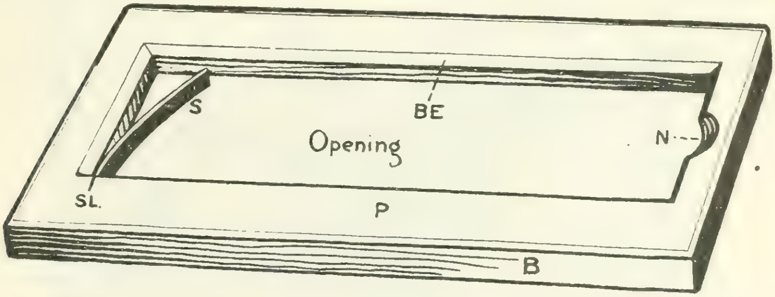


FIG. 2. Slide holder and protector. S, Clock spring; N, Notch to admit finger nail; BE Beveled edge of plate; B, Band about 5 mm. wide about plate; P, Plate 4 mm. thick.

This attachment is essentially a cover for the top of the incubator (Fig. 2), made of a plate of brass 4 mm. thick (P.), which has about its edge a band of 22 gauge brass 5 mm. wide (B) thus affording a rim at the lower edge of the plate 1 mm. in depth (R, Fig. 3). This rim fits snugly down over the top of the incubator, gentle pressure being required to seat the plate in position, where it is held by friction. An opening (Fig. 2) is cut through the plate large enough to admit an ordinary microscopic slide, which, when in place, rests on the top of the incubator (S, Fig. 3), closing the upper opening of the incubation chamber. The edges of the plate about this opening are beveled (BE, Fig. 2), so as not to hinder the turning of the objectives. At one end, the end opposite from where the slide is inserted, a curved piece of clock spring (S, Fig. 2) is fastened by soldering it into a small slit (Sl.) cut with a thread saw. This spring holds the slide firmly in place during the examination of any object, and in removing the slide, after the end opposite the spring has been released by raising with the finger nail, this spring forces the slide out of the opening to a point where it may be easily picked up with the fingers. The instrument is nickelplated.

The chief advantages of the device are that it holds the slide firmly in position, enabling the operator to observe a small object, such as a single amoeba, as long as desired without danger of the slide being moved about accidentally. Forming a sort of shallow well for the slide, it seals any little spaces between the edges of the slide and the top of the incubator, and protects the slide from cross currents of air. The added weight to the incubator is useful when operated on a plain microscope stage where there are no clips to hold the instrument. The thermal capacity of the attachment aids to some extent in maintaining a uniform temperature.

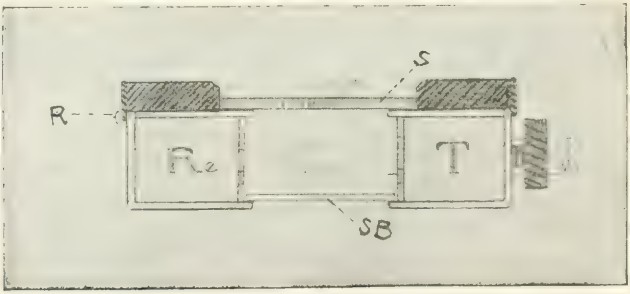


FIG. 3. Cross section of incubator and slide-holder. T, Thermostat element; Re, Resistance coil; S- Regular microscopic slide; R, Rim which fits over incubator; SB, Slide at bottom incubating chamber.

The attention of laboratory workers is called to this little device in the belief that it is a valuable addition to the C. S. & E. No. 8 Incubator, when used as a warming stage, and that the advantages afforded by it would be appreciated by any one who uses one of these warming stages. It is so simple in construction and so easily made, that any one possessing only slight ability as a craftsman may make one for himself with the aid of a few common tools.

*Department of Experimental Medicine.*

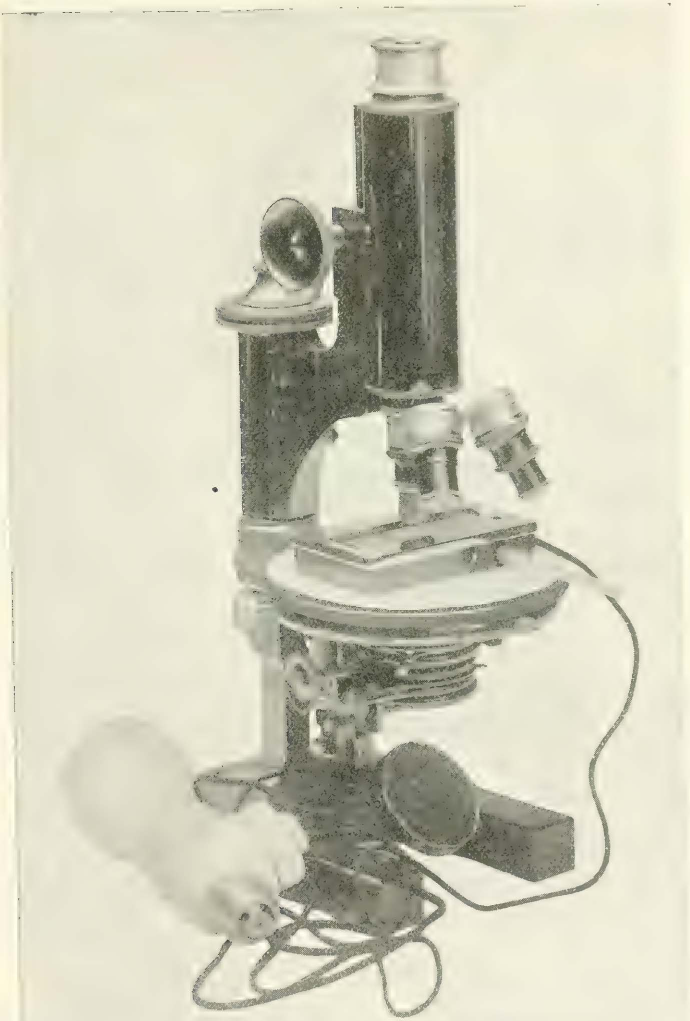


FIG. 4. Showing instrument in position with slide inserted.

# ON THE DETERIORATION OF CRUDE INDIAN CANNABIS.

C. R. ECKLER, Ph. C., M. S. AND F. A. MILLER, M. S.

[Read before the Scientific Section of the American Pharmaceutical Association at Indianapolis, Aug. 1917, and reprinted from the Journal of the A. Ph. A., Vol. VI, p. 872,]

It has long been known that crude Indian Cannabis loses its activity quite rapidly, and Marshall (1) and others have shown that the deterioration is due to oxidation of the active principles, but the rate of deterioration during commercial storage has not been determined and this was of particular interest to us. For the purpose of learning something on this point, two sets of experiments were carried out, our intention being to imitate the different conditions under which the crude drug might be kept. One lot of drug was stored in a cool basement in three portions—one portion sealed in alcohol, one portion sealed dry, and one portion dry unsealed. Another lot was stored in a warm attic in four portions—one portion, ground, sealed; one portion, ground, unsealed; one portion, whole, sealed; one portion, whole, unsealed.

## METHOD OF EXTRACTING SAMPLES.

All samples were finely ground and made into fluidextracts according to the U. S. P. method except that no heat was used in the process, the final percolate being evaporated under an air jet.

## METHOD OF TESTING.

The method of assay on pure bred fox terriers, essentially as described by us previously (2), was employed in this work. In all cases the drug was administered in the form of a fluidextract.

## EXPERIMENTS ON BASEMENT-STORED DRUG.

A lot of drug, supposed to be of the 1911 crop, was received December 28, 1911. The assay of this drug was finished February 14, 1912, and it was found to possess approximately 75 per cent. of standard activity. It was set aside for aging May 28, 1912, in a cool basement, as follows:

One portion of 69 pounds of ground drug was sealed in an alcohol barrel with enough alcohol to keep it well moistened.

One portion of 74 pounds whole drug, was sealed in an alcohol barrel dry.

One portion of 74 pounds, whole drug, was left unsealed in the original box in which it was received.

These portions were assayed February 14, 1917, and gave results as follows:

The sample sealed in alcohol seemed not to have lost appreciably in activity.

Both dry portions seemed to have lost fully 60 per cent. of their original activity. (The drug at this date possessed from 25 to 30 per cent. standard activity.) No difference could be noticed between the activity of the dry portions.

### EXPERIMENTS ON ATTIC-STORED DRUG.

A lot of drug was received December 27, 1912, claimed by the drug merchant to be of the 1912 crop. The assay of this drug was finished January 14, 1913, and it was found to possess approximately standard activity. March 17, 1913, four portions were set aside for aging, each portion consisting of ten 500 gram packages, as follows:

One portion, ground for percolation, in muslin bags.

One portion, ground for percolation, in amber bottles with paraffined corks.

One portion, whole drug, in muslin bags.

One portion, whole drug, in amber bottles with paraffined corks.

These portions were stored in an attic room of the laboratory where the temperature varied from 65° to 105° F., the average temperature for fall, winter, and spring being about 75° F., and for the warm summer months about 90° to 95° F.

It was our intention to assay these samples at least once each year, but the pressure of other work hindered to such an extent that we made, aside from the original testing, only three assays over a period of about 50 months. A carefully prepared fluidextract made from the same drug was used for comparison. This fluidextract was preserved in two well-filled, one-pint, amber bottles with paraffined corks, until the first aging period was over, that is, until the aged samples were assayed for the first time. After that time, the fluidextract was kept in well-filled, one-ounce, amber bottles with paraffined corks. These small bottles were placed in a glass cupboard in the laboratory, and a fresh one was opened for each succeeding assay.

March 31, 1914. First assay of aging samples. (Approximately 14½ months from date of first assay.) At this time no decided deterioration could be noticed with certainty. A few of the results suggested a very slight lessening of activity.

December 14, 1915. Second assay of samples. (Approximately 35 months from date of first assay.) All samples had apparently lost about 60 per cent. of their original activity. No decided difference could be noticed between them. The results on three out of eight dogs seemed to be slightly in favor of the sealed samples.

April 4, 1917. Third assay of samples. (Approximately 50½ months from date of first assay.) All samples seemed to have lost considerably over 90 per cent. of their original activity, in fact ten times the originally active dose caused not more than barely perceptible symptoms in some of the dogs, while in the others no decided effects were noticeable.

The fluidextract used for comparison still compared favorably with recently prepared fluidextracts from fresh drug which were considered of standard strength.

## REMARKS AND CONCLUSIONS.

From the results of the tests on the attic-stored samples, the loss in activity was practically 100 per cent. in about 50 months. (The drug at the end of the aging period was, however, about 55 months old from date of harvest.) This would give an average loss in activity of about 2 per cent. per month. Apparently, however, the deterioration did not proceed so rapidly at first, for in the first period of about 14 months not more than a very slight deterioration was noticeable, while during the next period of about 21 months there was a deterioration of nearly 60 per cent. of the original activity, and during the last period of about 15 months there was apparently a loss of approximately 40 per cent.

The dry samples stored in the basement lost in about 60 months, approximately 60 per cent. of their original activity, or about 1 per cent. each month on the average. (This drug at the end of the aging period was about 65 months old from date of harvest.) These results in connection with those of the preceding paragraph, would seem to suggest that the warm temperature of the attic was influential in increasing the rate of deterioration.

Drug stored in sealed containers in the dry state did not retain its activity appreciably longer than when stored in unsealed containers, nor did it retain its activity appreciably longer when stored whole than when ground.

Ground drug, moistened with alcohol and stored in a tight barrel, seemed to retain its full activity for at least 60 months.

*References.*

(1) Marshall. Experiments on the Cause of the Loss of Activity of Indian Hemp. Pharmaceutical Jr., Vol. 82, p. 418 (1909).

(2) Eckler and Miller. A Study of American Grown Cannabis in Comparison with Samples from Various Other Sources. Eighth International Congress of Applied Chemistry, Vol. 17, p. 23.

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# PHARMACOLOGICAL STUDIES OF THE IPECAC ALKALOIDS AND SOME SYNTHETIC DERIVATIVES OF CEPHAELINE

## I. STUDIES ON TOXICITY*

A. L. WALTERS, B. S., M. D. AND E. W. KOCH, A. M., M. D.

Three alkaloids have been obtained from ipecac, the root of either Cephaelis Ipecacuanha or Cephaelis acuminata. These are emetine, cephaeline and psychotrine. While it is claimed that other alkaloids have been isolated from ipecac, these findings have not been confirmed by subsequent investigations.

From an analysis of 145 different lots and samples of ipecac representing in all many thousand pounds of drug, our records show that commercial ipecac averages 2.17 per cent. of total alkaloids. The lowest assay was 1.83 and the highest 2.84 per cent. The average amount of emetine is to that of cephaeline as 63 is to 37. There is little or no difference in alkaloidal strength between Rio and Carthagena ipecac and the partition of the emetine and cephaeline in the two species is in general the same. These figures are not entirely in harmony with those usually published which, however, were based on very few assays and were no doubt due to individual variations in the drug assayed.

Carr and Pyman have assigned to psychotrine the formula $C_{28}H_{36}N_2O_4$, to cephaeline $C_{28}H_{38}N_2O_4$, and to emetine $C_{29}H_{40}N_2O_4$. These formulas show the close chemical relationship between these alkaloids. Cephaeline is psychotrine plus $H_2$, and emetine is cephaeline plus $CH_2$, or methyl-cephaeline. Emetine and cephaeline produce similar pharmacological effects qualitatively but differ considerably in the intensity of their action. Both are bactericidal and amebacidal, and are irritant, emetic and toxic in small doses. They differ, however, in that emetine is stronger as an amebacide and protozoöcide and is weaker as an irritant, emetic or toxic agent.

Since methylating cephaeline to produce emetine is known to increase the amebacidal property and diminish the emetic,

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irritant and toxic effects, it was thought probable that ethers of cephaeline with the higher homologous alcohols might further enhance the therapeutic value of this alkaloid. Accordingly the following new ethers of cephaeline were prepared,—ethyl, propyl, iso-propyl, butyl, iso-butyl, tertiary-butyl, amyl, iso-amyl, and allyl. The hydrochlorides, hydrobromides, phosphates, sulphates and hydriodides of the majority of these were obtained. These cephaeline compounds were made by boiling cephaeline with sodium ethylate and the corresponding alkyl bromide or iodide, extracting the cephaeline ether, thus formed, with ether; concentrating the ether solution, acidifying and allowing the salt to crystallize, and purifying by recrystallization. The hydrobromides of this series are slightly soluble in cold water and readily soluble in hot water. The hydrochlorides are more soluble than the hydrobromides. The phosphates and sulphates are the most readily soluble and the hydroiodides are the least soluble.

Most of the pharmacological work of early investigators on the ipecac alkaloids was carried out with mixtures of emetine and cephaeline, rather than the pure alkaloids, and hence their quantitative results may be questioned. The alkaloids used in our experiments were prepared in the Chemical Research Laboratory and their identity and purity established.

## TOXICITY

In making the toxicity tests, the main purpose was to determine the relative killing power of these various derivatives of

| Drug | Subcutaneously, White Rats | Subcutaneously, Guinea Pigs | Intravenously, Rabbits |
|---|---|---|---|
| Psychotrine hydrochloride | 1.00+ | 0.2+ | |
| Cephaeline hydrochloride | 0.0065 | 0.008 | |
| Cephaeline methyl ether (emetine) HCl | 0.012 | 0.016 | 0.005 |
| Cephaeline ethyl ether HBr | 0.015 | | |
| Cephaeline propyl ether phosphate | 0.045 | | 0.007 |
| Cephaeline iso-propyl ether HCl | 0.045 | 0.050 | |
| Cephaeline n-butyl ether HCl | 0.025 | | |
| Cephaeline iso-butyl ether HCl | 0.030 | | |
| Cephaeline tertiary-butyl ether HBr | 0.030 | | |
| Cephaeline iso-amyl ether HBr | 0.060 | | 0.010 |
| Cephaeline allyl ether HBr | 0.020 | | |

cephaeline. The animals used were white rats, guinea-pigs and rabbits. The preceding table gives a summary of the results obtained, the doses being gram of alkaloidal salt per kilogram of animal.

The most striking fact brought out by these figures is the decrease in toxicity as the higher alcohols are combined with cephæline. The addition of a methyl group to cephæline to make cephæline methyl ether (emetine) has decreased the toxicity one-half, and by replacing this methyl group by an iso-amyl group the toxicity is only one-fifth that of the cephæline methyl ether hydrochloride or about one-tenth as toxic as cephæline hydrochloride itself.

Another remarkable feature, considering the close chemical relation, is the very low toxicity of psychotrine. Even when given in a dose as large as 1 gram per kilogram of animal this alkaloid was not fatal to white rats. The minimum lethal dose for this substance was not determined. Smaller lethal doses have been published, but these may have been due to a contamination of the material as it is rather difficult to obtain psychotrine free from the other alkaloids of ipecac.

It will also be noted that the difference in toxicity between emetine hydrochloride and cephæline propyl ether phosphate or the iso-amyl ether hydrochloride when given intravenously to rabbits is not nearly so great as when these substances are given subcutaneously to rats or guinea-pigs. When given subcutaneously emetine hydrochloride is five times as toxic as cephaeline iso-amyl ether hydrobromide, but when given intravenously it is not more than twice as toxic. This is probably due in part to the difference in their rate of absorption from the subcutaneous tissues as other experiments to be described later will tend to prove.

It is noteworthy that the cephæline compounds containing the higher alcohol radicals decrease in toxicity, reaching the lowest degree of toxicity in cephaeline iso-amyl ether. It is also to be remarked that all the cephæline derivatives kill slowly. In the above experiments the animals were observed for at least seven days, and a death within that time was taken into account in determining the lethality. The minimum lethal dose (M.L.D.) usually killed in two to four days. The protocol on the following page will illustrate the method.

The subject of the cumulative poisonous action of emetine has been suggested and some work along this line has been done by Dale (1). We have found that one-half the minimal lethal dose, repeated at daily intervals subcutaneously, is quite uniformly fatal when a dose totaling two and one-half times the minimal lethal dose has been given. Thus the minimal lethal dose of emetine hydrochloride for rabbits is 10 mgm. per kilogram when given in a single subcutaneous injection; whereas 5 mgm. per kilogram injected daily will kill after the fifth dose or a total of 25 mgm. per kilogram. Cephæline iso-amyl ether hydrochloride, which is one-fifth as toxic as emetine hydrochloride when given subcutaneously in a single injection, bears this same relation when given in comparable sublethal dose at daily intervals. Thus four daily injections of 30 mgm. per kilogram, or five daily injections of 25 mgm. per kilogram each, cause death of the animal. Giving cephæline iso-amyl ether hydrochloride in daily injection of 5 mgm. per kilogram the rabbit succumbs as a rule after the twenty-second dose; that is, after a total of 120 mgm., which again is five times the fatal dose of emetine hydrochloride when given in daily injection of 5 mgm. per kilogram. These toxicity figures would indicate then that the cephæline derivatives

*Cephaeline propyl ether phosphate*

| Weight of Rat | Dose Per Kilogram | Injected | Result | Remarks |
|---|---|---|---|---|
| *gm.* | | | | |
| 174 | 0.035 | 11–12–1915 | Survived | Observed 10 days |
| 123 | 0.035 | 11–20–1915 | Survived | Observed 7 days |
| 141 | 0.035 | 10–28–1915 | Died | On tenth day |
| 186 | 0.040 | 11–12–1915 | Survived | Observed 10 days |
| 166 | 0.040 | 11–20–1915 | Survived | Observed 10 days |
| 110 | 0.040 | 10–30–1915 | Died | On ninth day |
| 140 | 0.040 | 12– 3–1915 | Died | On eighth day |
| 140 | 0.040 | 12– 3–1915 | Survived | Observed 10 days |
| 139 | 0.045 | 11– 2–1915 | Died | On second day |
| 194 | 0.045 | 11–12–1915 | Died | On third day |
| 176 | 0.050 | 11–12–1915 | Died | On second day |
| 119 | 0.050 | 11– 4–1915 | Died | On fourth day |

0.0075 gram in each cubic centimeter.
Minimal Lethal Dose = 0.045 gram per kilogram.
Injected into subcutaneous tissues of abdomen.

when given in small daily doses, one-tenth to one-twelfth their minimal lethal dose, will eventually prove toxic and that the period necessary to produce death in rabbits is about three weeks. Smaller doses than these may prove fatal over longer periods of time, but no experiments to demonstrate this were made.

It is evident that the animals of different genera do not succumb to exactly the same dose of these alkaloids and it is clearly impossible to make an exact analogy between these toxic doses and those for man. No authentic case of fatal poisoning in man from a single dose of emetine has been recorded. There has, however, been a fatality reported by Rowntree and Levy (2) in which emetine hydrochloride was administered daily for nineteen days to a man weighing 69.5 kgm. The total amount given was 1.74 grams, an average daily dose of 90 mgm. or 1.3 mgm. per kilogram. This corresponds closely with the toxic dose of emetine for rabbits, which we find to be 1 mgm. per kilogram given daily for about three weeks. These figures give some basis for estimating a single toxic dose for a man of 70 kgm. which then, by analogy, would be 0.7 gram or 10.8 grains.

Another death from emetine injections is reported by Dr. J. A. Snell (3). The patient was a girl 5 years old, who received 10.6 grains (688 mgm.) in twenty-one days, an average of 33 mgm. per day. Assuming the child's weight to have been 17 kgm, the fatal daily dose was 2 mgm. per kilogram.

Capt. Howard H. Johnson and Lieut. John A. Murphy (4) report two deaths in their service as probably due to emetine. One patient, a man of 22 years, received emetine as follows: August 10 to 19, 9 grains; August 21 to 24, 3 grains; September 5 to 15, 10 grains; September 27 to October 3, 3 grains; a total of 25 grains in 44 days. The second, a man of 38 years, received 1 grain of emetine a day for twelve and one-half days and then, after an interval of five days, was given ½ grain daily for fourteen days followed by 1 grain daily for four days, making a total of 23½ grains in thirty-five days. The body weight is not given in either case. An unexpected finding in these two cases at necropsy was the absence of inflammation of the alimentary tract which in fact was normal in appearance throughout its extent.

In this connection it may be of interest to review some of the published cases in which large doses of emetine have not been fatal. Johnson and Murphy (loc. cit.) report five cases showing toxic manifestations attributable to large doses of emetine. These doses in the five cases were as follows: 18 grains in twenty-six days, 29 grains in thirty-four days, 25⅔ grains in twenty-eight days, 22 grains in twenty-two days, and 22 grains, over a period not stated, to a patient who at some previous time had received a course of 23 grains. All these cases showed lassitude, weakness and extreme exhaustion. Neuritis, muscular tremor, rapid pulse and diarrhea were present in nearly all. Spehl and Collard (5) report the case of a man, age 28, who received 60 mgm. daily for six days, and then 90 mgm. a day for six days longer, making a total of 900 mgm. in a period of twelve days, or 75 mgm. per day. Assuming that this man weighed 65 kilograms he received somewhat more than 1 mgm. per kilogram daily. Severe symptoms followed the above course of treatment but the patient gradually recovered during two weeks from date of onset. Eshleman (6) reports the case of a man who received 1 grain, twice a day for five or six days. This patient developed a severe peripheral neuritis and a purpuric eruption of the skin. Allan (7) reports having given 2.4 grains of emetine hydrochloride followed on the succeeding day by an injection of 4 grains. The patient became nauseated and vomited once, four and a half hours after this injection. No other ill effects are mentioned. This is the largest single subcutaneous dose we have found recorded. Lyons (8) reports a man having taken 1 1-3 grains a day for sixteen days, a total of 21 1-3 grains. This patient showed general muscular weakness and a mild peripheral neuritis. There are many case reports where 1 grain of emetine has been given daily for twelve days with no ill effects.

## DISCUSSION.

From these experiments and case reports it would seem that the toxicity of emetine has generally been over-stated. The toxic dose of 10 grains is certainly a rather large one for an active alkaloid. The difference between the accepted therapeutic dose and the toxic dose is fairly large, since as a rule the former does not exceed one grain daily for ten or twelve days, whereas the latter is probably not less than one grain a day for twenty-one days. The more usual dosage of one-half to one grain of emetine per day for six or eight days is certainly on the safe side unless the patient shows an unusual susceptibility to the drug. However, the onset of general lassitude and depression with intensified diarrhea, lessened reflexes and perhaps a neuritis are indications which appear in sufficient time to prevent continued treatment and serious results. The very few fatalities among the many thousands of cases treated with emetine do not indicate it to be a dangerously toxic drug. The real danger, as has been shown, lies in the too long continued use of therapeutic doses, an entirely unnecessary procedure in the treatment of amebiasis as the active endamebas will be destroyed by half or one grain doses in six to twelve days, and the encysted forms, if present, will not be destroyed by continued emetine injections.

Since much has been written in an editorial way on the variation in the toxicity of emetine, and since most of the statements have been based on the article by Rowntree and Levy (2), it may not be out of place to comment on this subject. This paper by Rowntree and Levy was not on the toxicity of emetine, but on the variation in toxicity of commercial preparations, ampoules and tablets, of this alkaloid. Some of the editorials (9), however, misconstrued the statements of the article in question and dwelt at length on the variation in the toxicity of emetine and spoke of "impure emetine" and of "associated" or "dangerous impurities." As a matter of fact there are no associated impurities in ipecac which would increase the toxicity of emetine to anything like the extent indicated, for if all of the alkaloid should be cephæline, the only other active constituent of ipecac, it would be only twice as toxic as pure emetine and would in no way account for the published results. It should be remarked in commenting on this article, that the fatality reported was not caused by the preparation of emetine, claimed to be highly toxic, and that the patient, to whom this preparation was given, in very small dosage, did not exhibit symptoms which necessarily need be credited to the emetine. The most likely explanation of the author's experimental results would seem to be more logically explained by a variation in susceptibility to emetine shown by dogs of various breeds and sizes, as were probably used in these experiments. An examination of table 1 in Rowntree and Levy's article will show that some of the dogs, receiving the so-called "unusually toxic preparation" A, required a total of 2.85 mgm. per kilogram to kill, whereas others required 11.7 mgm. per kilogram, a variation in toxicity of over 400 per cent. when the same preparation was used. Still more contradictory is the claim for unusual toxicity of the preparation A, when the five commercial preparations were given to cats. If A is the same preparation found so toxic for dogs it is the least toxic of all when given to cats, requiring an average total of 30.25 mgm. per kilogram to kill. Furthermore, when given to cats, there was less variation between the average toxic doses of the five commercial preparations (21.07 to 30.25 mgm. per kilogram) than there was in the variation among four cats receiving the same preparation, which in one instance (E) ranged from 14 to 27.24 mgm. per kilogram.

It need only be pointed out that emetine is a definite, relatively stable alkaloid and that any increase in its toxicity can only be caused by an admixture of cephæline which, even in large amounts, will not dangerously heighten the toxicity, or else by an abnormally low content of water of crystallization, which will cause only a slight increase in toxicity. Any real increase in toxicity of commercial emetine preparations can only be due to a mistake in compounding, which can be determined much better by a chemical analysis than by a biological test.

## CONCLUSIONS.

The substitution of the methyl group in emetine by radicals of the higher homologous alcohols markedly decreases the toxicity. Cephæline iso-amyl ether is the least toxic of the series examined, being only one-fifth as toxic as emetine when given subcutaneously.

Emetine is not a very toxic alkaloid when given in a single dose, but is dangerous when given repeatedly in small doses over a considerable period of time.

# II. STUDIES ON EMETIC EFFECT AND IRRITANT ACTION.*

A. L. WALTERS, B. S., M. D., C. R. ECKLER, Ph. C., M. S.
AND E. W. KOCH, A. M., M. D.

## A. EMETIC EFFECT.

Both emetine and cephaeline are known to be emetic in small doses when taken by mouth. Since emetine is cephaeline methyl ether and is less emetic than cephaeline, it was of interest to determine the relative emetic power of some of the higher homologues of this series. The following ethers of cephaeline were prepared and their emetic action compared with that of cephaeline and emetine: ethyl, propyl, butyl, isoamyl and allyl. Cats were chosen as suitable animals for these emetic tests. Full grown cats were fed milk about 7:30 a. m. At 10:00 a. m. each cat received 1 ounce of fresh raw beef, finely cut. Between 11:30 and 12:00 the alkaloidal salt was given in solution by stomach tube, the solution with the washings amounting to 20 cc. The animals were kept under observation until 5:30 p. m. The same animal could not be used satisfactorily oftener than once a week. After continued use the animals became more susceptible to the emetic effect of the drug. As different cats varied considerably as to the amount of drug required to produce emesis, it was necessary to give at least two of the alkaloids to the same animal in order to obtain a correct comparison. This difference in reaction and the increasing susceptibility on repeated dosage necessitated the use of a large number of animals. In the experiments summarized here, fifty-six cats were used and the period of experimentation extended over several months. The following protocols illustrate the method pursued, but conclusions must be based on a series of such tests.

*Protocols*

| Animal | Drug | Dose Per Cat | Result |
|---|---|---|---|
| | | *gram* | |
| 92 | Cephaeline hydrochloride | 0.002 | Vomited |
| | Cephaeline hydrochloride | 0.002 | Vomited |
| | Emetine hydrochloride | 0.004 | Did not vomit |
| | Emetine hydrochloride | 0.004 | Did not vomit |
| 86 | Cephaeline hydrochloride | 0.002 | Vomited |
| | Cephaeline hydrochloride | 0.002 | Did not vomit |
| | Cephaeline hydrochloride | 0.002 | Did not vomit |
| | Emetine hydrochloride | 0.005 | Vomited |
| | Emetine hydrochloride | 0.004 | Did not vomit |
| | Emetine hydrochloride | 0.004 | Did not vomit |

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*Protocols—Continued.*

| Animal | Drug | Dose Per Cat | Result |
|---|---|---|---|
| | | *gram* | |
| 12 | Cephaeline hydrochloride | 0.004 | Vomited |
| | Cephaeline hydrochloride | 0.003 | Did not vomit |
| | Cephaeline hydrochloride | 0.003 | Did not vomit |
| | Cephaeline hydrochloride | 0.002 | Did not vomit |
| | Cephaeline propyl ether hydrobromide | 0.016 | Vomited |
| | Cephaeline propyl ether hydrobromide | 0.012 | Did not vomit |
| | Cephaeline propyl ether hydrobromide | 0.010 | Did not vomit |
| | Cephaeline ethyl ether hydrobromide | 0.012 | Vomited |
| | Cephaeline butyl ether hydrobromide | 0.012 | Vomited |
| | Emetine hydrochloride | 0.010 | Vomited |
| 85 | Emetine hydrochloride | 0.008 | Did not vomit |
| | Emetine hydrochloride | 0.012 | Vomited |
| | Cephaeline propyl ether phosphate | 0.012 | Did not vomit |
| | Cephaeline propyl ether phosphate | 0.016 | Did not vomit |
| | Cephaeline propyl ether phosphate | 0.020 | Vomited |
| 13 | Emetine hydrochloride | 0.008 | Did not vomit |
| | Emetine hydrochloride | 0.010 | Did not vomit |
| | Emetine hydrochloride | 0.012 | Vomited |
| | Cephaeline iso-amyl ether hydrobromide | 0.024 | Did not vomit |
| | Cephaeline iso-amyl ether hydrobromide | 0.030 | Did not vomit |
| | Cephaeline iso-amyl ether hydrobromide | 0.036 | Vomited |
| 31 | Cephaeline allyl ether phosphate | 0.015 | Did not vomit |
| | Cephaeline allyl ether phosphate | 0.020 | Vomited |
| | Emetine phosphate | 0.010 | Did not vomit |
| | Emetine phosphate | 0.012 | Vomited |
| | Psychotrine hydrochloride | 0.075 | Did not vomit |
| | Psychotrine hydrochloride | 0.100 | Vomited |
| | Psychotrine hydrochloride | 0.100 | Did not vomit |
| | Psychotrine hydrochloride | 0.120 | Did not vomit |
| | Psychotrine hydrochloride | 0.130 | Did not vomit |

A total of 290 doses were given and from an analysis of the several experiments the following comparative figures were compiled, the emetic dose of cephaeline having been taken as 1.

| | |
|---|---|
| Cephaeline hydrochloride | 1. |
| Cephaeline methyl ether (emetine) hydrochloride | 2. |
| Cephaeline ethyl ether hydrobromide | 2.4 |
| Cephaeline propyl ether hydrobromide | 3.6 |
| Cephaeline propyl ether phosphate | 3.8 |
| Cephaeline butyl ether hydrobromide | 3.6 |
| Cephaeline iso-amyl ether hydrobromide | 5.0 |
| Cephaeline allyl ether phosphate | 2.5 |

The results are only approximately correct at best, but they show that the emetic power of this series of compounds diminishes much in the same order as does their toxicity (15) and that emetine hydrochloride is about one-half as emetic as cephaeline hydrochloride and that cephaeline iso-amyl ether hydrobromide is only one-fifth as emetic as cephaeline hydrochloride.

It was noted that some salts of these cephaeline compounds were more emetic than others, and accordingly the hydriodides of the methyl, propyl and iso-amyl derivatives were tried in a manner similar to that above detailed, with the exception that the alkaloidal salts were all given in capsules instead of in solution.

The following tables are two of a series of seven and demonstrate the greatly diminished emetic power of the difficultly soluble hydriodides of the cephaeline propyl and iso-amyl ethers. Emetine hydriodide was not appreciably weaker as an emetic than emetine hydrochloride.

*Cat 14; weight, 3086 grams*

| Date | Drug | Dose Per Cat | Results |
|---|---|---|---|
| | | *gram* | |
| April 5, 1916 | Cephaeline propyl ether hydriodide | 0.020 | Vomited |
| April 12, 1916 | Cephaleine propyl ether hydriodide | 0.015 | Did not vomit |
| April 19, 1916 | Cephaeline propyl ether hydriodide | 0.020 | Vomited |
| April 27, 1916 | Cephaeline propyl ether hydriodide | 0.015 | Did not vomit |
| May 5, 1916 | Emetine hydrochloride | 0.010 | Vomited |
| May 12, 1916 | Emetine hydrochloride | 0.007 | Vomited |
| May 19, 1916 | Emetine hydrochloride | 0.005 | Did not vomit |
| May 26, 1916 | Emetine hydrochloride | 0.005 | Vomited |
| June 2, 1916 | Emetine hydriodide | 0.007 | Vomited |

*Cat 18; weight, 2484 grams*

| | | | |
|---|---|---|---|
| March 30, 1916 | Cephaeline iso-amyl ether hydriodide | 0.040 | Did not vomit |
| April 14, 1916 | Cephaeline iso-amyl ether hydriodide | 0.050 | Did not vomit |
| April 21, 1916 | Cephaeline iso-amyl ether hydriodide | 0.060 | Did not vomit |
| April 28, 1916 | Cephaeline iso-amyl ether hydriodide | 0.060 | Did not vomit |
| May 5, 1916 | Cephaeline iso-amyl ether hydriodide | 0.065 | Did not vomit |

*Cat 18; weight, 2484 grams—Continued.*

| Date | Drug | Dose Per Cat | Results |
|---|---|---|---|
| | | *gram* | |
| May 12, 1916 | Cephaeline iso-amyl ether hydriodide | 0.070 | Did not vomit |
| May 19, 1916 | Cephaeline iso-amyl ether hydriodide | 0.075 | Did not vomit |
| May 26, 1916 | Emetine hydrochloride | 0.010 | Did not vomit |
| June 2, 1916 | Emetine hydrochloride | 0.012 | Vomited |
| June 15, 1916 | Cephaeline iso-amyl ether hydriodide | 0.080 | Did not vomit |
| June 23, 1916 | Cephaeline iso-amyl ether hydriodide | 0.085 | Did not vomit |
| July 7, 1916 | Cephaeline iso-amyl ether hydriodide | 0.090 | Did not vomit |
| July 21, 1916 | Cephaeline iso-amyl ether hydriodide | 0.095 | Vomited |

A summary of the results with these seven cats is given below.

| Cat | Drug | Emetic Dose | Drug | Emetic Dose |
|---|---|---|---|---|
| | | *mgm.* | | *mgm.* |
| 14 | Cephaeline propyl ether hydriodide | 15–20 | Emetine hydrochloride | 5 |
| 17 | Cephaeline propyl ether hydriodide | 35–40 | Emetine hydrochloride | 10–12 |
| 19 | Cephaeline propyl ether hydriodide | 35–40 | Emetine hydrochloride | 10–12 |
| 15 | Cephaeline iso-amyl ether hydriodide | 55–60 | Emetine hydrochloride | 7–10 |
| 16 | Cephaeline iso-amyl ether hydriodide | 75–80 | Emetine hydrochloride | 10–12 |
| 18 | Cephaeline iso-amyl ether hydriodide | 90–95 | Emetine hydrochloride | 10–12 |
| 20 | Cephaeline iso-amyl ether hydriodide | 60–65 | Emetine hydrochloride | 12–15 |

From these tests made on seven animals, cephaeline propyl ether hydriodide is not more than one-third as emetic for cats as is emetine hydrochloride, and cephaeline iso-amyl ether hydriodide is not more than one-sixth as emetic as emetine hydrochloride or one-twelfth as emetic as cephaeline hydrochloride, the mother substance.

A few tests on persons showed an individual variation in susceptibility to the ipecac alkaloids. As a rule one-eighth grain of cephaeline hydrochloride and one-fourth grain of emetine hydrochloride proved emetic. One-sixth grain of cephaeline iso-amyl ether hydrochloride caused slight nausea but no emesis in two persons and no noticeable effect in four others; one-third grain caused slight nausea in one case and no effect in another, and one person, a boy of sixteen years, took one-sixth grain three times a day for fourteen days without any symptoms of vomiting or nausea. The cephaeline iso-amyl ether hydriodide was given in single doses of three-fourths of a grain without producing nausea. One person took one-fourth grain of this hydriodide three times a day for fourteen days without nausea resulting. Larger doses than the above were not given.

### B. IRRITANT ACTION.

The irritant action of emetine hydrochloride when injected hypodermatically has been noted by all who have used this alkaloidal salt. Its irritant effect has also been evident where accidentally solutions of it have come in contact with the conjunctiva or with the mucous membrane of the oral cavity. Furthermore, workers employed in the manufacture of emetine or in handling powdered ipecac root not infrequently develop a cutaneous eruption as a result of the local action of the drug. A noticeable feature of this irritating action is the slowness of its onset. When injected hypodermatically the pain or soreness does not develop for several hours, sometimes not until the second day. When solutions of emetine hydrochloride, or even particles of the alkaloid itself, come into contact with the conjunctiva, pain is not felt for six or eight hours and severe inflammation does not set in previous to this time.

#### *Conjunctival tests.*

In order to determine the relative irritating effects of cephaeline and some of its derivatives, solutions of these alkaloidal salts were instilled into the eyes of rabbits. It was determined that one-tenth per cent. solutions were well suited for this purpose. The alkaloidal salts were dissolved in 0.85 per cent. sodium chloride solutions and the right conjunctival sac was filled with the fluid to be tested. This was held in the sac for one minute and then allowed to flow out naturally. The left eye was treated in a similar manner with 0.85 per cent, salt solution and was used as a control. The condition of the ocular and palpebral conjunctiva was observed from time to time over several days. The following protocol illustrates this method.

| Time | Rabbit | Agent | Palpebral Conjunctiva | Ocular Conjunctiva |
|---|---|---|---|---|
| 9.43 | 1 | 0.1 per cent cephaeline propyl ether phosphate | Normal appearance | Normal appearance |
| 9.45 | 2 | 0.1 per cent emetine hydrochloride. | Normal appearance | Normal appearance |
| After 3 hours | 1 | | Injected | Injected |
| | 2 | | Injected | Injected |
| After 7 hours | 1 | | Injected | Injected |
| | 2 | | Injection greater than rabbit 1 | Injected |
| After 23 hours | 1 | | Injection slight | Normal |
| | 2 | | Marked injection and increased secretion. Edges of lids reddened. Much worse than rabbit 1 | Injected |
| After 49 hours | 1 | | Normal | Normal |
| | 2 | | Still markedly injected, less than at 23 hours | Injected |
| After 97 hours | 1 | | Normal | Normal |
| | 2 | | Injection still present | |
| After 121 hours | 1 | | Normal | Normal |
| | 2 | | Normal | Normal |

Thirty-two tests of this nature were made using cephaeline hydrochloride, emetine hydrochloride, cephaeline propyl ether phosphate, cephaeline iso-propyl ether hydrochloride and hydrobromide, cephaeline iso-amyl ether hydrochloride and hydrobromide, and cephaeline allyl ether hydrobromide. Of these substances cephaeline hydrochloride and emetine hydrochloride were undoubtedly the most irritating to the conjunctiva. The irritation was intense and long lasting. Of the other substances the cephaeline iso-amyl ether was the least irritating, although the difference among these was not very great.

These experiments were not carried further as at this time the fallacy of the results was discovered. On the assumption that cephaeline iso-amyl ether hydrochloride was considerably less irritating than emetine hydrochloride, ½ grain of this substance in 1 cc. of sterile water was injected subcutaneously into the deltoid region of one of the laboratory workers. This injection was followed by a similar one on each of the two succeeding days and on the fourth day a deep intramuscular injection of ½ grain was given. The intramuscular injection gave rise to only a mild reaction. The injections given subcutaneously gave rise to considerable local reaction which consisted of a reddening of the skin and a soreness which developed during the first twenty-four hours. These effects increased during the following forty-eight hours at the end of which time there was also some induration. Objectively from seventy-two to ninety-six hours there was improvement though subjectively the condition was said to be worse. At the end of ninety-six hours the soreness decreased too. The reaction gradually decreased and was practically cleared up at the end of two weeks, although a small indurated subcutaneous nodule could still be palpated at the site of one of the injections.

Injections of ½ grain cephaeline iso-amyl ether hydrochloride were made intramuscularly in a second individual. The left arm received an injection into the triceps on the first day and was only slightly tender on the second day, was swollen, tender and painful on the third day, and the inflammation was rapidly subsiding on the fourth day. On the second day, an injection was made into the right triceps. This showed only slight tenderness on the following day and another injection was made into the left arm, this time into the deltoid. This last injection gave only slight evidence of irritation. The right arm was not sore on the day following the injection nor on the second day but on the morning of the third day the patient wakened with a very sore arm and felt ill. This being Sunday the patient was not seen until the fourth day when he presented a swollen, slightly reddened and very tender right arm. The swelling began at the point of injection just above the deltoid insertion and extended over the triceps and outer aspect of the arm to the elbow. There was no soreness or swelling above the point of injection. The patient could not raise the arm. The swelling gradually subsided and the pain decreased so that the arm was practically normal at the end of the tenth day following the injection.

Three other patients who received injections of this preparation for amebic dysentery complained of considerable soreness at the sites of the injections.

These clinical tests seemed to conclusively prove that the conjunctival tests for irritation as carried out were not a reliable measure of the irritation afforded by hypodermatic injections. While emetine hydrochloride often causes severe local reaction when injected, we have never seen it cause as severe reactions as were produced by the cephaeline iso-amyl ether hydrochloride. Further laboratory tests which will now be described have substantiated the clinical findings, or rather have disproved the findings based on the conjunctival tests.

### *Intramuscular tests.*

Solution of the phosphate of each alkaloid was compared with a like solution of emetine phosphate. The emetine phosphate was injected deep into the right lumbar muscles of a rabbit and the substance to be tested into the left. These solutions in different experiments were given in amounts of either 0.25 cc. or 0.5 cc. The amount of alkaloid injected varied from 2 mgm. to 32.4 mgm. The injection of 32.4 mgm. into each side was occasionally fatal. The usual dilution was 16.2 mgm. in 0.5 cc., this being the concentration most commonly employed in clinical practice. The rabbits were chloroformed on the fourth day following the injection and the subcutaneous and intramuscular reactions of the two sides were carefully compared macroscopically. In these experiments the phosphates of the following cephaeline ethers were tested: methyl, ethyl, propyl, iso-propyl, butyl, iso-butyl, tertiary butyl, iso-amyl, and allyl.

Without going into detail to describe the differences in the intensity of reaction, the comparative irritating effects may be indicated as follows:

| Dose | Emetine Phosphate | |
|---|---|---|
| | | *Cephaeline phosphate* |
| 16.2 mgm. in 0.5 cc. | +++ | +++ |
| | | *Cephaeline ethyl ether phosphate* |
| 16.2 mgm. in 0.5 cc. | ++ | ++ |
| 32.4 mgm. in 0.5 cc. | ++ | ++ died during night |
| | | *Cephaeline propyl ether phosphate* |
| 32.4 mgm. in 0.5 cc. | ++ | +++ |
| 32.4 mgm. in 0.5 cc. | ++ | +++ |
| 16.2 mgm. in 0.5 cc. | ++ | +++ |
| 16.2 mgm. in 0.5 cc. | ++ | +++ |
| 16.2 mgm. in 0.25 cc. | +++ | ++ |
| 16.2 mgm. in 0.25 cc. | ++ | +++ |
| | | *Cephaeline iso-propyl ether phosphate* |
| 16.2 mgm. in 0.5 cc. | ++ | + |
| 16.2 mgm. in 0.5 cc. | + | + |
| 16.2 mgm. in 0.5 cc. | ++ | ++ |
| 32.4 mgm. in 0.5 cc. | ++ | ++ (died after 1 day) |
| | | *Cephaeline butyl ether phosphate* |
| 32.4 mgm. in 0.5 cc. | ++ | ++ |
| 32.4 mgm. in 0.5 cc. | +++ | ++ |
| 16.2 mgm. in 0.5 cc. | + | +++ |
| 16.2 mgm. in 0.5 cc. | ++ | ++ |
| | | *Cephaeline iso-butyl ether phosphate* |
| 32.4 mgm. in 0.5 cc. | +++ | ++ |
| 16.2 mgm. in 0.5 cc. | +++ | ++ |
| | | *Cephaeline tertiary butyl ether phosphate* |
| 32.4 mgm. in 0.5 cc. | ++ | + |
| 16.2 mgm. in 0.5 cc. | + | ++ |
| | | *Cephaeline iso-amyl ether phosphate* |
| 32.4 mgm. in 0.5 cc. | ++ | +++ |
| 16.2 mgm. in 0.5 cc. | ++ | +++ |
| 16.2 mgm. in 0.5 cc. | +++ | ++++ |
| 16.2 mgm. in 0.5 cc. | ++ | +++ |
| | | *Cephaeline allyl ether phosphate* |
| 16.2 mgm. in 0.5 cc. | +++ | +++ |
| 16.2 mgm. in 0.25 cc. | +++ | ++ |
| 16.2 mgm. in 0.5 cc. | ++ | +++ |
| 16.2 mgm. in 0.5 cc. | ++ | ++ |

Slight inflammation (+), severe inflammation (++), very severe inflammation (+++), necrosis (++++).

As may be seen, the various derivatives were not tested against each other in the same rabbit, but each was tested against emetine phosphate, so that these results only in a general way indicate the relative irritating effect of the alkaloids tested. Suffice it to say that the cephaeline iso-amyl ether phosphate, which was least irritating in the conjunctiva tests, was the most irritating when given intramuscularly. Cephaeline and all of its derivatives tested by this method were quite irritating when given in the above dilutions and none of them possessed any decided advantage over emetine in this respect.

We have personally injected doses of ½ grain cephaeline iso-amyl ether hydrochloride in 1 cc. in four patients and have had reports of four other cases in which this drug was used. In all cases severe pain and local inflammation was caused. We have made from three to six injections of ½ grain cephaeline propyl ether hydrochloride or phosphate at daily intervals in twelve patients and have reports from similar injections in several others and in none of them was there more than slight local soreness at the point of injection. In three of these patients emetine hydrochloride was also injected for comparison and no difference could be noted in its local action and that of cephaeline propyl ether hydrochloride.

It may be that the apparently lessened irritating effect of these derivatives of cephaeline when tested on the rabbit's eye and their lowered toxicity when given subcutaneously to rats and guinea pigs is due to their being more difficultly absorbed from the tissues. Those compounds difficult of absorption would be washed out of the eye before seriously affecting the conjunctiva and when injected intramuscularly these compounds would be more irritating on account of their remaining longer in contact with the tissues. The further fact that their toxic dose bears an entirely different relation to emetine when given intravenously and when given subcutaneously would tend to confirm the above assumption.

In connection with the irritating action of the alkaloids of ipecac, we would like to record some incidental observations on the action of powdered ipecac root in producing "ipecac asthma." In our laboratories there are seven men in whom typical bronchial asthmatic attacks occur whenever they come in contact with ipecac dust in the air. On days when ipecac is being ground in the drug mills these men, although they may be one or two buildings distant from the grinding room, will suffer from asthma. In some cases this attack is so severe as to temporarily incapacitate them. In some the attacks are mild and pass off after a short time in the open air. In others the attack may be mild at first and then become more severe at night after the patient has gone home and the effects may be noticed for as long as a week. Six of these cases have never had asthma at any other time or from any other cause. On the other hand certain individuals who are subject to asthma and who frequently come in contact with powdered ipecac are not affected by it. In four of the above cases adrenalin hydrochloride has been given hypodermatically and relieves the difficult breathing quite promptly. That this ipecac asthma is not due to the alkaloids is evident, for no one handling either cephaeline or emetine has ever made such complaint. Two of the men mentioned above are chemists and frequently come in contact with the alkaloids but are never affected by them. Powdered ipecac root, from which the alkaloids have been removed, is still capable of producing these asthmatic attacks in susceptible persons, so that this would seem to be another instance of vegetable protein sensitization.

## CONCLUSIONS.

These experiments have demonstrated that in cats the emetic dose of emetine hydrochloride is approximately twice that of cephaeline hydrochloride, and that the higher homologues of this series decrease in emetic power very much in the same ratio as they do in toxicity, as reported in a previous paper (15). Furthermore, it has been shown that the hydrochloride, hydrobromide and hydriodide of emetine vary only slightly in their emetic power, but that the hydriodide of cephaeline iso-amyl ether, due to its relative insolubility, is about one-half as emetic as the hydrobromide or hydrochloride of cephaeline iso-amyl ether and only one-sixth as emetic as emetine hydrochloride.

When tested on the conjunctiva of rabbits, emetine and cephaeline are the most irritant of this series and cephaeline iso-amyl ether is least irritating.

When injected intramuscularly in rabbits cephaeline iso-amyl ether is the most irritant while the difference between the other less irritant members of the series is not marked.

Cephaeline propyl ether phosphate gives no more than a slight local reaction when injected hypodermatically into persons, while cephaeline iso-amyl ether salts cause severe pain, soreness and local inflammation.

# III. STUDIES ON PROTOZOÖCIDAL AND BACTERICIDAL ACTION.*

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## A. AMEBACIDAL ACTION

Following the earlier work of Vedder (10) showing that emetine or ipecac is strongly amebacidal for water amebas, some experiments to compare the effects of emetine and cephaeline on these organisms were begun in 1913. Later the ethyl, propyl, butyl, amyl and allyl ethers of cephaeline were similarly tested.

The cultures were obtained by examining drops of a laboratory culture (obtained from Butler College) for amebas and when these were found the drop containing them was washed off the slide with 50 cc. of tap water into a small flask and 0.5 cc. of ordinary bouillon added. The flask was loosely stoppered with cotton and left at room temperature for several days or until a scum was noticed on the surface of the liquid. Active amebas were usually abundant in this scum at first but as a rule disappeared entirely from the culture in a week or ten days. As soon as amebas were found in the broth culture, loopfuls of this material were planted on the surface of agar plates and slant agar tubes. This agar was composed of agar, 20 grams, sodium chloride, 3 grams; beef extract, 3 grams, and water to make 1000 cc. It was 1 per cent. alkaline to phenolphathalein.

The amebas multiplied quite readily at room temperature on this medium as long as it remained moist, but went into the quiescent stage when it became dry. It was found advisable to transfer the culture to fresh plates every four or five days in order to have actively motile organisms.

Numerous tests were made by placing a loopful of culture rich in amebas into a drop of emetine or cephaeline hydrochloride solution on a microscope slide and sealing a cover glass on with vaseline to prevent evaporation. Controls in water were observed.

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*Protocol.* Culture from plate 11 containing very numerous actively motile amebas. One loopful of this culture was placed in a drop of 1:50,000 solution of emetine hydrochloride and observed immediately, using a 4 mm. objective.
11.00 a.m. Two active amebas in the field.
11.10 a.m. Still active.
11.25 a.m. Still active.
11.30 a.m. Non-motile. Round in outline.
11.45 a.m. Non-motile. Round in outline.
11.48 a.m. One of them has disintegrated.
11.54 a.m. The second has disintegrated.
12.00 m. No other amebas found on searching the slide.

A loopful of the same culture placed in a 1:50,000 cephaeline hydrochloride solution showed disintegration of the amebas in the same time (fifty-four minutes).

In 1:10,000 solutions emetine and cephaeline hydrochloride were both actively amebacidal and destroyed all motile amebas in seven to twenty minutes.

In 1:50,000 solutions, emetine acted more rapidly than cephaeline in a series of tests and appeared to be more amebacidal. In greater dilutions (1:100,000) the results over short periods were inconstant and were not recorded.

In another series of experiments, bouillon cultures of amebas and solutions of emetine hydrochloride and cephaeline hydrochloride were mixed in definite proportions and allowed to stand in test tubes for one hour, after which agar plates were inoculated from them. Forty-eight hours later control plates contained numerous active amebas while no amebas were found in the plates inoculated from the emetine or cephaeline hydrochloride solutions of 1:50,000, 1:100,000 and 1:200,000 strength. These same plates were examined four days later and all plates then contained active amebas.

This experiment was repeated using 1:200,000, 1:300,000 and 1:500,000 solutions of emetine and cephaeline hydrochloride and exposing the amebas to these solutions in test tubes for four hours. Plates inoculated from these solutions contained active amebas three days later.

This experiment was again repeated using 1:200,000 solutions and exposing the amebas to them for seven hours. Eight days later active amebas were present on all plates inoculated from these solutions.

To fifty hour cultures of amebas on agar slants were added

5 cc. of 1:50,000 solutions of emetine hydrochloride and cephaeline hydrochloride and these allowed to stay in contact for two hours. The solutions were then poured off and transplants made from the surface of the slant onto agar plates. All these plates contained amebas five days later.

In another series of experiments a drop of the drug solution of the desired dilution was placed on a slide and the ameba culture stirred into it, the exact time of the mixing being noted. The cover slip was then sealed in position and the preparation examined for amebas. If any were found they were carefully observed in order to note any changes they might undergo as a result of being acted upon by the drug. The amebas used in this series were grown in tap water to which beef extract had been added in the proportion of 1 part in 25,000. The drugs used were dissolved in distilled water. Proper controls were observed adding the cultures to distilled water. In this series emetine and the ethyl, propyl, and iso-butyl ethers of cephaeline were tested for amebacidal action. The following illustrates the method:

Dilution of Emetine:

1:1000. Six non-motile amebas were seen. No motility or change in shape occurred during twenty minutes.

1:5000. One ameba broke up in six minutes, another in nineteen minutes. Ten other non-motile amebas were seen.

1:5000. Two large, non-motile amebas were seen and remained motionless for tweny-five minutes.

1:8000. One ameba broke up at end of twenty-two minutes, seventy-five other non-motile organisms were seen.

1:10,000. Seven amebas were seen, all slugishly active. One ballooned out in eight minutes, another in seventeen, a third in twenty, a fourth in twenty-three, a fifth in twenty-five, and a sixth in forty. The seventh was still sluggishly active after forty minutes.

1:12,000. Ten slides were examined and thirteen amebas seen. These all ballooned out within two hours, most of them within thirty minutes. Very few of them showed much activity.

1:35,000. Ten amebas were seen and all were actively motile at the end of fifteen minutes.

The results of this series may be thus stated, emetine hydrochloride and cephaeline propyl ether hydrobromide in dilutions of 1:1000, 1:5000, 1:8000, 1:10,000 and 1:12,000 were effective in killing some of the amebas, while the changes induced in other amebas were of such a nature that it was hard to decide whether they were dead or merely in a resting or resistant stage. Neither of the above two drugs in 1:35,000 cause the amebas to cease their motility within fifteen minutes. Cephaeline iso-butyl ether hydrochloride and cephaeline ethyl ether hydrochloride in 1:10,000 dilution killed some amebas and in others induced changes that made the endpoint questionable while still other amebas remained active for thirty minutes.

A second similar series was carried out in which the amebas were obtained from agar plates and in which the drug solutions were made up to 1:1000 solutions in 0.85 per cent. sodium chloride and were then made up to the desired dilution with tap water. Emetine hydrochloride and cephaeline iso-amyl ether hydrobromide were used in dilutions of 1:1000, 1:5000, 1:10,000 and 1:15,000. Some of the amebas were broken up within a few minutes, others remained round and quiescent, while some were sluggishly active for considerable periods of time. One slide containing amebas in emetine hydrochloride, 1:1000, showed at the end of an hour, 26 non-motile round amebas and 5 very sluggishly active ones. In a 1:5000 solution of emetine hydrochloride 22 slightly active amebas were found at the end of three hours. In a slide containing 1:10,000 emetine hydrochloride, 20 sluggishly active amebas were seen at the end of one hour. In a 1:15,000 solution of emetine hydrochloride 25 fairly active amebas were seen after one hundred and sixty-five minutes exposure. One slide of a 1:15,000 solution was examined seventeen hours after preparation. Sixty-five non-motile round forms and the disintegrated remains of many amebas were seen. No motile amebas were present.

The following are observations made with cephaeline iso-amyl ether hydrobromide, given somewhat in detail but omitting many similar slides studied. See table on page 327.

In discussing these results it must be admitted that the effect of the ipecac alkaloids on water amebas varies greatly. In some cases the amebas are quickly destroyed as evidenced by their complete disintegration, in others they became round and remain quiescent for long periods when it is impossible to say whether they are dead or not, and in still others they remain sluggishly active for a long time. It is possible to have these three events take place in amebas from the same culture

| Dilution | Slide | Remarks |
|---|---|---|
| 1:10,000 | 1 | One round form was seen. The cytoplasm disappeared at the end of fifteen minutes leaving only the nuclear material and granular debris. Two active amebas were seen and these were still sluggishly active at the end of one hour |
| 1:10,000 | 2 | One round form was observed. At end of seven minues the cell wall and cytoplasm suddenly disappeared leaving only the granular and nuclear material. The slide was examined again after seventy minutes, but only the disintegrated remains of amebas could be found |
| 1:10,000 | 3 | One ameba sluggishly changed its outline for fifteen minutes and then assumed a round form. During the next ten minutes some slight changes in outline occurred. The organism was quiescent during the following hour |
| 1:10,000 | 4 | No active amebas were present but quite a few round forms were seen. One of these broke up in fifteen minutes. Some granular material alone remaining. In searching the slide other disintegrated amebas were seen |
| 1:15,000 | 5 | One sluggishly changed its outline for five minutes and then assumed a round form. It remained quiet for three minutes and then disintegrated. Another one sluggishly changed its outline for fifty minutes, then assumed a round form, was quiet for seventeen minutes, and then broke up completely. No actively motile ones were seen at any time |
| 1:15,000 | 6 | Four amebas sluggishly changing their outlines were seen. One broke up in three minutes, another in six, another in nine and the fourth in fifty-seven minutes. Many disintegrated ones were found on searching the field at the end of the hour. No progressively motile ones were seen at any time |
| 1:15,000 | 7 | One changed its location slightly, and its outline sluggishly for thirty-one minutes and then assumed a round form. The granular material gradually became contracted leaving a clear zone all around it. No other changes were seen during the following sixty minutes |
| 1:15,000 | 8 | Many amebas were found. They changed their locations very little and their outlines sluggishly. At the end of five hours many were still present; some were round, some irregular in outline and some broken up; none were motile |
| 1:15,000 | 9 | One was active for two minutes, then assumed a round form and broke up after one minute. Another round form broke up four minutes after the slide was made |
| 1:15,000 | 10 | One round form broke up in two minutes and another in five. No active ones were seen |
| 1:15,000 | 11 | One round form broke up in three minutes. Six other very small amebas of a different species were sluggishly active after twenty-two minutes |

and even on the same slide. The process of disintegration does not always occur, and is really the only readily apparent indication of death. Disintegration is initiated by a motile organism first becoming non-motile, assuming a round form, gradually swelling up or "ballooning out" and finally breaking up by rupturing and scattering particles of protoplasm in all directions. Other organisms which do not rupture may become coarsely granular but as there are all degrees of this, it cannot be taken as an indication of death.

Our experiments extended over a considerable period of time and it is possible that we dealt with different species of amebas. However, in general morphology they all appeared the same with the exception of one very small ameba which was undoubtedly a different species and which was more resistant to the alkaloids, and was disregarded in our experiments.

It seems evident from our plating experiments that some of the organisms are encysted and thus resist the action of emetine and cephaeline. For example cultures on agar were not destroyed in two hours by 1:50,000 solutions nor in seven hours by 1:200,000 solutions, although no active forms could be found immediately after this treatment and it required three or four days for them to develop on the agar plates. Our results are not strictly comparable with those of Vedder (10) who planted his cultures in a 5 per cent. bouillon to which he added his ipecac solutions and allowed them to act for twenty-four, forty-eight and seventy-two hours. His dilutions however, were remarkably high, 1:20,000 solution of F. E. Ipecac containing not more than 1:1,000,000 of the ipecac alkaloids, emetine and cephaeline.

Wherry (11) tried the action of emetine on water amebas growing with symbiotic bacteria and concluded that emetine in 1:20,000, 1:100,000 and 1:200,000 dilutions killed the amebas after twenty-three and one-half hours exposure but did not kill them in one hour.

One thing that has impressed us in all our work with these alkaloids is the fact that they act slowly. This is evident in their toxic, irritant, bactericidal and other actions. Minimal lethal doses cause death only after two to four days as a rule. Irritation when caused by placing emetine in the human eye does not manifest itself objectively or subjectively for four to eight hours. Bactericidal effects are more evident when the drug solution is allowed to act on the bacteria for a considerable time. So, too, the amebacidal effect is exerted slowly and while a very high dilution of these alkaloids will kill amebas when allowed to act over a long period, a much stronger solution fails to kill in a brief time. That this failure is not due entirely to encystment is shown by the maintenance of motility by these organisms while in contact with the alkaloids in solution.

### B. ENDAMEBACIDAL EFFECTS.

The slow and irregular amebacidal effect of the ipecac alkaloids in vitro was further confirmed by their action on the Endameba buccalis obtained from the pus of pyorrhea lesions. In these experiments a drop of normal salt solution, containing the alkaloidal salt in the desired dilution, was placed on a microscope slide and into it was stirred a very small amount of pus taken from the bottom of a pyorrhea lesion. The cover slip was then sealed on with vaseline and the slide on a warm stage placed under the microscope and examined for endamebas. The effect of the drug was then observed and notes made as shown in the following tables.

| Emetine HCl | Slide | Remarks |
|---|---|---|
| 1:20,000 | 1 | One endameba sluggishly active for twenty-seven minutes when it assumed a round form and remained thus during the next thirty minutes |
| 1:20,000 | 2 | One active endameba assumed a round form in twenty minutes and remained thus for thirty minutes |
| 1:20,000 | 3 | One actively motile endameba was still active at end of one hour. At this time four other motile ones were found |
| 1:20,000 | 4 | One was actively motile at end of one hour |
| 1:20,000 | 5 | Six were actively motile at the end of sixty-five minutes |
| 1:10,000 | 6 | Three actively motile ones were observed for one hour. The slide was then examined and eleven other actively motile ones were found |
| 1:10,000 | 7 | Seven actively motile ones were seen eighty minutes after preparing the slide |
| 1:5,000 | 8 | One actively motile endameba observed for thirty minutes |
| 1:5,000 | 9 | One actively motile, assumed a round form in twenty minutes and remained thus for the ten minutes observed. Many similar round forms but no active ones were seen |
| 1:5,000 | 10 | One sluggishly active for twelve minutes when it became round and motionless |

| Emetine HCl | Slide | Remarks |
|---|---|---|
| 1:5,000 | 11 | One actively motile endameba assumed an irregular form in fifteen minutes and remained thus unchanged for thirty minutes longer. It then became actively motile again and was still motile at the end of seventy-five minutes from the time the slide was prepared. Two other actively motile ones were found at the end of eighty minutes |
| 1:1,000 | 12 | Two endamebas were found. They were still actively motile after eighty-five minutes. One actively motile one came to rest in twenty minutes and during the following eleven minutes gradually assumed a round form. In looking over the field eighty-four minutes from the time of preparation, seven actively motile endamebas were seen |
| 1:1,000 | 13 | Twelve actively motile endamebas were seen at the end of ninety minutes. A few round forms were also seen |
| 1:500 | 14 | Two actively motile ones were seen. They came to rest after eight minutes and remained thus during an hour |
| 1:500 | 15 | One actively motile for one hour |
| 1:100 | 16 | One actively motile endameba was seen. Gradually became more sluggish and at the end of sixty-five minutes assumed a round form. This round form could still be seen after twelve hours |
| 1:100 | 17 | One endameba assumed round form in seven minutes and then remained unchanged during the following ten minutes of observation. A second endameba was actively changing its form and rather sluggishly its position at the end of one hour. A third endameba was changing its shape at the end of sixty-five minutes |
| 1:50 | 18 | One sluggishly active endameba was seen and assumed a round form in five minutes and remained unchanged at end of fifteen minutes |
| 1:50 | 19 | No motile endamebas found |
| 1:50 | 20 | No motile endamebas found |

| Cephaeline Propyl Ether Phosphate | Slide | Remarks |
|---|---|---|
| 1:20,000 | 1 | One actively motile endameba found after seventy minutes. Many round forms seen |
| 1:20,000 | 2 | One sluggishly active for thirty minutes, then became round. Another sluggishly active for forty minutes then became round. Both remained so at end of one hour |
| 1:20,000 | 3 | One was sluggishly motile for thirty-three minutes and then remained quiet during an hour |
| 1:12,000 | 4 | Two sluggishly motile endamebas were killed in fifty-three minutes. Another showed sluggish motility for seventy-three minutes |
| 1:12,000 | 5 | Four were sluggishly motile during an hour |

| Cephaeline PropylEther Phosphate | Slide | Remarks |
|---|---|---|
| 1:12,000 | 6 | Five were sluggishly motile during forty minutes observation |
| 1:10,000 | 7 | Eleven actively motile endamebas were seen after one hour |
| 1:10,000 | 8 | Fourteen were still active at end of seventy-five minutes |
| 1:5,000 | 9 | One active at end of fifty minutes |
| 1:5,000 | 10 | One sluggishly active for one hour |
| 1:5,000 | 11 | One activly motile for three hours |
| 1:5,000 | 12 | One sluggishly motile for thirty minutes then became round |
| 1:5,000 | 13 | One actively motile for twenty-two minutes then became round |
| 1:5,000 | 14 | One, actively motile, was observed for an hour. The field was then searched and four other actively motile ones were found. One of these was still motile at the end of three hours |
| 1:500 | 15 | One active endameba came to rest and assumed a round form in twenty minutes and remained unchanged during another twenty minutes of observation |
| 1:100 | 16 | Eleven different slides were examined and in none were actively motile endamebas seen. In four of the slides some endamebas were found which were slightly and slowly changing their outline but not their position. These also became quiescent within a few minutes |
| 1:50 | 17 | No active endamebas were found |

| Cephaeline Iso-Amyl Ether Hydrobromide | Slide | Remarks |
|---|---|---|
| 1:20,000 | 1 | One actively motile endameba came to rest in five minutes and remained so for fifteen minutes. During the next twenty minutes it changed its outline but not its location. Forty minutes from the time the slide was prepared this endameba became actively motile again and continued so during the remainder of the observation period of seventy minutes. In looking over the field sixty to seventy minutes after the slide was prepared sixty actively motile organisms were counted |
|  | 2 | Two endamebas were actively motile at the end of eighty-five minutes |
| 1:1,000 |  | Four slides were examined but no motile endamebas seen. Round forms were present |

These results are in harmony with those of Kolmer and Smith (12) who found that dilutions of emetine as strong as 1 in 800 did not kill the endameba of pyorrhea within two hours. These authors, however, state that when the action of emetine on endamebas is observed over longer periods (six hours) amebacidal effect is evident in dilutions as high as 1:400,000. As our object was to compare the various cephaeline derivatives and as this method did not lend itself well to that purpose we did not attempt to confirm Kolmer and Smith's findings, but decided to test the relative protozoöcidal effects of these cephaeline derivatives on paramecia.

It may be well to add that the high endamebacidal action of emetine in weak dilution is well proven by clinical experience in administering this drug in endamebic dysentery and endamebic pyorrhea; and yet there are some instances in these diseases where prolonged courses of emetine fail to eradicate the endamebas from the lesions. Most cases, however, respond promptly to treatment and the refractory ones are probably due to encystment of the organisms.

We have tested the endamebacidal action of emetine and cephaeline adsorbed with Lloyd's Reagent (Alcresta Tablets of Ipecac) given orally and in most cases have caused complete disappearance of endamebas from pyorrhea lesions by giving an equivalent of 60 grains of ipecac a day for six days. This is in accord with the findings of Bass and Johns (13). We have also tried with similar results, emetine hydrochloride, cephaeline propyl ether phosphate, and cephaeline iso-amyl ether hydrochloride given hypodermatically in daily doses of ½ grain. Cephaeline iso-amyl ether hydrochloride was too irritating to use in any number of cases, but its endamebacidal effect was very evident in one case, who was given ½ grain daily for four days. On the first day 819 actively motile endamebas were counted in two slides made from the pus on the point of one applicator. The organisms rapidly decreased in number and on the fifth day none could be found in the two slides examined. On the seventh day six slides were examined but careful search failed to reveal any endamebas.

### C. ACTION ON PARAMECIA.

Vedder (10) noted the highly toxic action of emetine on paramecia and Sir Leonard Rogers (14) also used paramecia as test organisms. Rogers says:

> In the first place, I found that the cephaeline salt had a less energetic action on pathogenic amoebae in fresh dysenteric stools than the emetine salt had. It is however, difficult to obtain accurate comparisons of the lethal action of the two alkaloids in this way because dysenteric amoebae cannot be cultivated, and they tend to die out in dysenteric stools within a few hours. Moreover, when the amoebae cease moving in the presence of the alkaloid, if it is not of sufficient strength to cause changes in the structure of the organism visible under the microscope, it is impossible to say if the organism has been killed or has entered a quiescent stage, perhaps preparatory to becoming encysted.
>
> . . . . I therefore tried the effects of the alkaloids on the common water paramecia, and found that high dilutions of the salts of both emetine and cephaeline caused these organisms to rapidly lose their motility, while many of them presented clear round spaces which soon became large bladder-like protrusions. When the solutions were added to water containing a large number of paramecia, they rapidly lost their movement and fell to the bottom of the tubes, and this change corresponded with the microscopical ones just mentioned and could be easily followed with a hand lens.
>
> It does not necessarily follow that the action of these alkaloids on paramecia is exactly parallel with that on the amoebia dysenterica, yet the fact that I have found them to be in close agreement in their actions on these two forms of protozoa is suggestive of such a relationship and makes the results of my experiments worthy of record.

Paramecia are well adapted to this purpose as their death point is readily determined. Normally they are in constant motion, their cilia moving so rapidly as to be invisible when observed with a 6 mm. objective. As the ipecac alkaloid begins to affect the paramecia, they progress more slowly, their cilia move less rapidly and can readily be seen and the paramecia come to rest. Their cilia continue to move slowly and finally stop completely. If the organisms are observed a little longer, their bodies become further swollen and will as a rule abruptly rupture and disintegrate.

Paramecia are easily cultivated in tap water containing one gram of beef extract in 25,000 cc. In order to keep the organisms multiplying, this medium is changed or added to about once a week. Increased multiplication occurs on the addition of a few bread crumbs. The cultures used in our work had been propagated in the laboratory for two years.

In agreement with previous workers, we found that paramecia were sensitive to slight changes in their environment, so that they differed from day to day in their susceptibility to

*Paramecia culture, 61, August 21*

| Cephaeline Hydro-Chloride | Emetine Phosphate | Cephaeline Ethyl Ether Phosphate | Cephaeline Propyl Ether Phosphate | Cephaeline Iso-Propyl Ether Phosphate | Cephaeline Butyl Ether Phosphate | Cephaeline Iso-Butyl Ether Phosphate | Cephaeline Tertiary Butyl Ether Phosphate | Cephaeline Iso-Amyl Ether Phosphate | Cephaeline Allyl Ether Phosphate |
|---|---|---|---|---|---|---|---|---|---|
| 1: 20,000<br>2: 13<br>2: 28 – 15 | 1: 20,000<br>1: 54<br>2: 03 – 9 | 1: 20,000<br>2: 25<br>2: 35 – 10 | | 1: 40,000<br>2: 42<br>2: 54 – 12 | 1: 40,000<br>2: 54<br>2: 57 – 3 | 1: 40,000<br>2: 17<br>2: 21 – 4 | 1: 40,000<br>3: 02½<br>3: 06 – 3½ | | 1: 20,000<br>3: 22½<br>3: 28 – 5½ |
| 2: 13<br>2: 32 – 19 | 1: 54<br>2: 06 – 12 | 3: 37<br>3: 45 – 8 | | 2: 42<br>3: 00 – 18 | 2: 54<br>2: 59 – 5 | 2: 17<br>2: 21 – 4 | 3: 15<br>3: 19½ – 4½ | | 3: 22½<br>3: 28 – 5½ |
| 4: 04<br>4: 26 – 22 | 3: 26<br>3: 42 – 16 | 4: 08½<br>4: 18 – 9½ | 1: 60,000<br>2: 28<br>2: 39 – 11 | 4: 27½<br>4: 41 – 13½ | 4: 30½<br>4: 34 – 3½ | | | | 5: 19½<br>5: 32½ – 13 |
| 4: 04<br>4: 26 – 22 | 3: 26<br>3: 39 – 13 | | 4: 23½<br>4: 34 – 10½ | 4: 27½<br>4: 42 – 14½ | | | | | 5: 19½<br>5: 33 – 13½ |
| | | | 4: 23½<br>4: 33 – 9½ | | 1: 80,000<br>4: 42<br>4: 51 – 9 | 1: 80,000<br>4: 53<br>5: 01 – 8 | 1: 80,000<br>5: 01<br>5: 12 – 11 | 1: 80,000<br>3: 08½<br>3: 13 – 4½ | |
| | | | | | 4: 42<br>4: 50 – 8 | 4: 53<br>5: 02 – 9 | 5: 01<br>5: 12 – 11 | 3: 08½<br>3: 11 – 2½ | |
| | | | | | | | | 1: 160,000<br>5: 09<br>5: 18 – 9 | |
| | | | | | | | | 5: 09<br>5: 15 – 6 | |

*Paramecia culture 69, August 28*

| Emetine Phosphate | Cephaeline Propyl Ether Phosphate | Cephaeline Iso-Propyl Ether Phosphate | Cephaeline Butyl Ether Phosphate | Cephaeline Iso-Butyl Ether Phosphate | Cephaeline Tertiary Butyl Ether Phosphate |
|---|---|---|---|---|---|
| 1: 40,000<br>9: 43<br>10: 27 — 44 | 1: 40,000<br>9: 33½<br>9: 39 — 5½ | 1: 40,000<br>9: 39<br>9: 51 — 12 | 1: 40,000<br>2: 44½<br>2: 49 — 4½ | 1: 40,000<br>2: 52<br>2: 58 — 6 | 1: 40,000<br>3: 01½<br>3: 07½ — 6 |
| 9: 43<br>10: 12 — 29 | 9: 33½<br>9: 43½ — 10 | 9: 39<br>9: 53 — 14 | 2: 44½<br>2: 49 — 4½ | 2: 52<br>2: 58 — 6 | 3: 01½<br>3: 11 — 9½ |
| 9: 55<br>10: 30 — 35 | 10: 37½<br>10: 45½ — 8 | 10: 41<br>10: 57 — 16 | | | |
| 10: 49<br>11: 23 — 34 | 10: 37½<br>10: 51½ — 14 | 10: 41<br>10: 54 — 13 | 1: 60,000<br>3: 27½<br>3: 34½ — 7 | 1: 60,000<br>3: 31½<br>3: 40½ — 9 | 1: 60,000<br>3: 38½<br>3: 47½ — 9 |
| 10: 49<br>11: 51 — 62 | 10: 57<br>11: 11 — 14 | 1: 03<br>11: 15 — 12 | 3: 27½<br>3:35 — 7½ | 3: 31½<br>3: 43 — 11½ | 3: 38½<br>3: 50 — 11½ |
| 1: 57<br>2: 33 — 36 | 10: 57<br>11: 03 — 6 | 11: 03<br>11: 23 — 20 | 3: 46½<br>3: 54 — 7½ | 3: 59<br>4: 06 — 7 | 4: 04<br>4: 18 — 14 |
| | 11: 25½<br>11: 34 — 8½ | 11: 34<br>11: 57 — 23 | 3: 46½<br>3: 51 — 7½ | 3: 59<br>4: 07 — 8 | 4: 04<br>4: 13 — 9 |
| | 1: 55<br>2: 06 — 11 | 11: 34<br>11: 54 — 20 | 4: 18½<br>4: 25 — 6½ | 4: 21<br>4: 31 — 13 | 4: 34½<br>4: 46½ — 12 |
| | 1: 55<br>2: 02 — 7 | 2: 10<br>2: 25 — 15 | 4: 18½<br>4: 26 — 7½ | 4: 21<br>4: 32½ — 11½ | 4: 34½<br>4: 48 — 13½ |
| | 2: 07½<br>2: 16 — 8½ | 2: 10<br>2: 34 — 21 | | | |
| | 2: 07½<br>2: 15 — 7½ | | | | |
| | 4: 52½<br>5: 03 — 10½ | | | | |
| | 4: 52½<br>5: 01½ — 9 | | | | |
| Average 40 min. | 10 min. | 17 min. | 4½ min.<br>1: 60,000 — 7¼ min. | 6 min.<br>1: 60,000 — 10 min. | 8 min.<br>1: 60,000 — 11½ min. |

| Date | Cephaeline Hydrochloride | Emetine Phosphate | Cephaeline Ethyl Ether Phosphate | Cephaeline Propyl Ether Phosphate | Cephaeline Iso-Propyl Ether Phosphate | Cephaeline N-Butyl Ether Phosphate | Cephaeline Iso-Butyl Ether Phosphate | Cephaeline Tertiary Butyl Ether Phosphate | Cephaeline Iso-Amyl Ether Phosphate | Cephaeline Allyl Ether Phosphate | Culture |
|---|---|---|---|---|---|---|---|---|---|---|---|
| August 8 | | 1: 20,000<br>$10\frac{1}{2}$ min. | | | | | | | 1: 100,000<br>3 min.<br>1: 200,000<br>8 min.<br>1: 300,000<br>11 min. | | 59 |
| 13 | | 1: 20,000<br>$16\frac{2}{3}$ min. | 1: 20,000<br>$9\frac{1}{2}$ min. | 1: 20,000<br>$2\frac{2}{3}$ min.<br>1: 40,000<br>4 min.<br>1: 80,000<br>11 min. | | | | | | | 60 |
| 15 | | 1: 20,000<br>53 min. | 1: 20,000<br>41 min. | 1: 20,000<br>23 min. | 1: 20,000<br>$16\frac{2}{3}$ min. | 1: 20,000<br>$6\frac{1}{2}$ min. | | | | | 61 |
| 16 | | 1: 20,000<br>30 min. | | 1: 20,000<br>12 min. | 1: 20,000<br>18 min. | 1: 20,000<br>10 min. | 1: 20,000<br>7 min. | 1: 20,000<br>12 min. | 1: 20,000<br>2 min. | 1: 20,000<br>17 min. | 61 |
| 17 | 1: 10,000<br>37 min. | 1: 10,000<br>32 min. | | | | 1: 40,000<br>9 min. | 1: 40,000<br>$8\frac{1}{3}$ min. | 1: 40,000<br>11 min.<br>1: 30,000<br>8 min.<br>1: 20,000<br>6 min. | 1: 40,000<br>5 min.<br>1: 60,000<br>$5\frac{2}{3}$ min.<br>1: 80,000<br>10 min.<br>1: 160,000<br>16 min. | | 61 |
| 20 | 1: 20,000<br>16 min.<br>1: 10,000<br>12 min. | 1: 20,000<br>11 min.<br>1: 10,000<br>8 min. | 1: 20,000<br>10 min. | 1: 20,000<br>3 min.<br>1: 40,000<br>6 min.<br>1: 60,000<br>11 min. | 1: 20,000<br>6 min.<br>1: 10,000<br>14 min. | 1: 10,000<br>4 min. | | | 1: 80,000<br>6 min. | | 61 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 21 | 1: 10,000<br>34 min. | 1: 20,000<br>33 min. | 1: 20,000<br>33 min. | 1: 60,000<br>36 min. | 1: 40,000<br>34 min. | 1: 40,000<br>21 min. | 1: 40,000<br>7 min. | 1: 40,000<br>15 min. | 1: 20,000<br>2 min.<br>1: 60,000<br>14 min.<br>1: 80,000<br>14 min. | 1: 20,000<br>31 min. | 62 |
| 21 | 1: 20,000<br>19 min. | 1: 20,000<br>12½ min. | 1: 20,000<br>9 min. | 1: 60,000<br>10 min. | 1: 40,000<br>14½ min. | 1: 40,000<br>4 min.<br>1: 80,000<br>8½ min. | 1: 40,000<br>4 min.<br>1: 80,000<br>8½ min. | 1: 40,000<br>4 min.<br>1: 80,000<br>11 min. | 1: 80,000<br>3½ min.<br>1: 160,000<br>7½ min. | 1: 20,000<br>9½ min. | 61 |
| 23 | | | | 1: 20,000<br>4 4-5 min. | 1: 20,000<br>5 3-5 min. | 1: 20,000<br>3 min.<br>1: 40,000<br>4½ min. | | | | | 62 |
| 24 | | | | 1: 20,000<br>6 min. | 1: 20,000<br>7 min. | | | | | | 62 |
| 28 | | 1: 40,000<br>40 min. | | 1: 40,000<br>10 min. | 1: 40,000<br>17 min. | 1: 40,000<br>4½ min.<br>1: 60,000<br>7¼ min. | 1: 40,000<br>6 min.<br>1: 60,000<br>10 min. | 1: 40,000<br>8 min.<br>1: 60,000<br>11½ min. | | | 69 |
| 29 | | 1: 20,000<br>59 min.<br>1: 40,000<br>62½ min. | | 1: 40,000<br>14 min.<br>1: 60,000<br>16¼ min.<br>1: 80,000<br>25½ min. | | 1: 40,000<br>6¼ min.<br>1: 60,000<br>12 min.<br>1: 80,000<br>11½ min. | | | 1: 40,000<br>5¼ min.<br>1: 60,000<br>7½ min.<br>1: 80,000<br>11½ min. | | 69 |
| Sept.<br>20 | | 1: 20,000<br>27 min. | | | | 1: 40,000<br>5⅔ min. | | | 1: 40,000<br>4⅔ min. | | 75 |
| 21 | | 1: 20,000<br>27 min. | | | | 1: 80,000<br>18 min. | | | 1: 80,000<br>13 min. | | 75 |

the action of emetine. In fact this variation may be remarkable as was exemplified by certain cultures tested on the same day. Culture 66 was killed by 1:2,000 solution of emetine phosphate within five minutes, whereas culture 67 was killed by the same solution only after four hours exposure. Culture 67 had been made four days previously by adding 100 cc. of culture 59 to 900 cc. of tap water containing 1:25,000 beef extract and some bread crumbs. Culture 66 had been similarly made from culture 59 four days previously but no bread was added. Usually the variations are much less than this, though they are frequently pronounced. Therefore we used a solution of emetine phosphate as a standard for comparison in determining the relative protozoocidal effect of each of the synthetic derivatives of cephaeline and also tested as many of them as possible on the same culture. All of these various alkaloids were tested as phosphates and their solutions made with tap water.

The method of procedure was to add by means of accurately graduated pipettes 0.5 cc. of the alkaloid solution to 0.5 cc. of the paramecia culture. These solutions were thoroughly mixed and then poured into two cells on specially constructed microscope slides, each cell having a capacity of 0.5 cc. Cover slips were then applied so that no air bubbles formed between the surface of the liquid and the cover slips. Each cell contained usually from 10 to 30 paramecia, the number depending on the richness of the culture used. The organisms could be observed easily with a 6 mm. objective and the exact time of their death noted. The time required to kill all the organisms in the cell furnished the figures for comparison; in other words, the most resistant paramecium in each cell was used as the test organism.

The tables on pages 334 and 335 show the tests made on two days, the 21st and 28th of August.

These tables illustrate the method used and show that individual paramecia from the same culture may vary considerably in their resistance to a given alkaloid, but that the comparative results of a number of tests are fairly concordant. The results of several series of such tests are summarized on pages 336 and 337.

Despite certain discrepancies appearing in the tabulation, it may be concluded that the higher homologues of this series are much more destructive to paramecia than either cephaeline or emetine. Indeed it would appear that cephaeline iso-amyl ether phosphate is fifteen to twenty times as effective as emetine phosphate, whether this be measured by the time required for equal dilutions to kill or by the dilution of each required to kill in the same length of time. Thus on August 16 and 21, emetine phosphate was effective in thirty and thirty-three minutes, whereas cephaeline iso-amyl ether phosphate killed paramecia from the same cultures in two minutes. On August 8, a dilution of 1:300,000 of this last named salt killed paramecia of culture 59 in eleven minutes, whereas a dilution of 1:20,000 of emetine phosphate killed them in ten and one-half minutes. Cephaeline hydrochloride is certainly less active in this respect than emetine phosphate while both the ethyl and allyl derivatives are somewhat more toxic to paramecia than is emetine. The propyl, butyl, and iso-amyl derivatives are certainly much more protozoocidal to these organisms. In order to determine the relative efficiency of these three they were compared on August 29 using culture 69 and again on September 20 and 21, using culture 75. From these results, recorded in the preceding table, it is evident that these derivatives increase in activity in the order named. We have previously shown (15) that the substitution of the methyl group in emetine by radicals of the higher homologous alcohols decreases the toxicity when given subcutaneously to animals and it is of interest to note that this substitution markedly increases the toxicity for these protozoa.

### D. BACTERICIDAL ACTION.

The experiments of Vedder (10) showed that fluid extract of ipecac has an antiseptic action or inhibitory effect on the growth of B. typhosus, B. paratyphosus, B. dysenteriae, and Staphylococcus aureus. Wherry (11) testing the effect of emetine on amebas growing with a symbiotic bacillus, noted that the emetine in a dilution of 1:20,000 killed this bacillus in forty-eight hours. Price (16) found that emetine in 1:10,000 solution inhibited the growth of bacterial organisms taken from pyorrhea pockets and also had marked germicidal action upon them. He also stated that cephaeline had a similar but somewhat weaker bactericidal action. Kolmer and

Smith (12), in a detailed study of the bactericidal action of emetine, concluded that emetine possesses bactericidal properties, but that prolonged contact with bacteria is required before this action becomes apparent. They worked with B. typhosus, B. anthracis, B. subtilis, Staphylococcus aureus and Streptococcus salivarius.

Our experiments were carried out on cultures of Staphylococcus aureus with the object of comparing the germicidal action of emetine phosphate with cephaeline propyl ether and cephaeline iso-amyl ether phosphates. The method of procedure and results follow:

To each of three tubes containing 10 cc. of broth was added 2 cc., 1 cc., 0.5 cc. and 0.1 cc. respectively of a 5 per cent. solution of each salt. Then 0.1 cc. of a twenty-four hour filtered staphylococcus aureus culture was added to each tube and mixed thoroughly, thus making dilutions of the alkaloidal salts of 1:121, 1:222, 1:424, and 1:2040. The tubes were incubated for twenty-four hours after which observations for growth were made. A transplant was made from each tube by streaking two loopfuls of the broth over the surface of a dextrose agar slant. This was repeated at twenty-four hour intervals for nine days. Control tubes were run by adding 0.1 cc. of the culture to each of three broth tubes. See pages 341 and 342.

Results obtained from the three control tubes indicate that after three days incubation the cultures lose vitality and eventually die. Emetine phosphate solutions of 1:121 are therefore not proven germicidal by these tests but apparently exert some inhibitory effect. Cephaeline propyl ether phosphate is germicidal in solutions of 1:222 and shows some inhibition in solutions of 1:424 for the first twenty-four hours but after that none. Cephaeline iso-amyl ether phosphate is germicidal in the weakest dilution used, namely 1:2040.

Additional tests on this last named substance were made in the same manner as before except that 20 cc. of broth and 1 per cent. solution of the salt were used. Two sets of control tubes, one containing 10 cc. and the other 20 cc. of inoculated broth, were observed and incidently the loss by evaporation was noted from day to day.

*Emetine phosphate*

| | One Day | | Two Days | | Three Days | | Four Days | | Six Days | | Seven Days | | Eight Days | | Nine Days | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar |
| *cc.*<br>2 | 3+ | 3+<br>Heavy | 3+ | 3+<br>Heavy | 3+ | 3+<br>2 – I. C.<br>1 – 2 C | 3+ | 3+<br>I. C. | 3+ | 1+ 2 –<br>1 – I. C. | 3+ | 1+ 2 –<br>1 – 1 C | 3+ | 3 – | 3+ | 3 – |
| 1 | 3+ | 3+<br>Heavy | 3+ | 3+<br>2 Heavy<br>1 – I. C. | 3+ | 3+<br>2 – I. C.<br>1 – 3 C | 3+ | 2+ 1 –<br>1 – I. C.<br>1 – 3 C | 3+ | 3 – | 3+ | 3 – | 3+ | 3 – | 3+ | 3 – |
| 0.5 | 3+ | 3+<br>Heavy | 3+ | 3+<br>Heavy | 3+ | 3+<br>I. C. | 3+ | 3+<br>I. C. | 3+ | 1+ 2 –<br>1 – 1 C | 3+ | 3 – | 3+ | 3 – | 3+ | 3 – |
| 0.1 | 3+ | 3+<br>Heavy | 3+ | 3+<br>Heavy | 3+ | 3+<br>I. C. | 3+ | 3+<br>I. C. | 3+ | 3+<br>1 – 1 C<br>1 – 7 C<br>1 – 9 C | 3+ | 1+ 2 –<br>1 – 12 C | 3+ | 2+ 1 –<br>1 – 1 C<br>1 – 1 C | 3+ | 3 – |

*Cephaeline propyl ether phosphate*

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – |
| 1 | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – |
| 0.5 | 3 – | 2 – 1+<br>1 – I. C. | 3 – | 1 – 2+<br>2 Heavy | 1 – 2+ | 1 – 2+<br>2 Heavy | 1 – 2+ | 1 – 2+<br>2 Heavy | 1 – 2+ | 2 – 1+<br>1 – I. C. | 1 – 2+ | 3 – | 1 – 2+ | 3 – | 1 – 2+ | 3 – |
| 0.1 | 3+ | 3+<br>Heavy | 3+ | 3+<br>2 Heavy<br>1 – I. C. | 3+ | 3+<br>1 – 3 C<br>2 – I. C. | 3+ | 3+<br>1 – 2 C<br>1 – 2 C<br>1 – 2 C | 3+ | 3 – | 3+ | 3 – | 3+ | 3 – | 3+ | 3 – |

*Cephaeline iso-amyl ether phosphate*

| | One Day | | Two Days | | Three Days | | Four Days | | Six Days | | Seven Days | | Eight Days | | Nine Days | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar |
| cc.<br>2 | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – |
| 1 | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – |
| 0.5 | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – |
| 0.1 | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – | 3 – |
| *Control tubes* | | | | | | | | | | | | | | | | |
| | 3 + | 3 +<br>Heavy | 3 + | 3 +<br>Heavy | 3 + | 3 +<br>I. C. | 3 + | 3 +<br>I. C. | 3 + | 2 + 1 –<br>I. C. | 3 + | 2 + 1 –<br>1 – 9 C<br>1 – 12 C | 3 + | 3 – | 3 + | 3 – |

Note: No observations made on 5th day. "C" indicates colonies; "I. C." indicates isolated colonies too numerous to count. "Heavy" means heavy growth.

*Cephaeline iso-amyl ether phosphate*

| | One Day | | Two Days | | Three Days | | Four Days | | Six Days | | Seven Days | | Eight Days | | Nine Days | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar |
| 2 cc. 1:1105 | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− |
| 1 cc. 1:2110 | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− |
| 0.5 cc. 1:4120 | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− | 3− |
| 0.1 cc. 1:20,200 | 3− | 2+1−<br>Heavy | 2+1− | 2+1−<br>Heavy | 2+1− | 2+1−<br>Heavy | 2+1− | 2+1−<br>1. C. | 2+1− | 3− | 2+1− | 3− | 2+1− | 3− | 2+1− | 3− |

*Control tubes*

| | One Day | | Two Days | | Three Days | | Four Days | | Six Days | | Seven Days | | Eight Days | | Nine Days | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar | Broth | Agar |
| 10 cc. | 3+ | 3+<br>Heavy | 3+ | 3+<br>Heavy | 3+ | 3+<br>Heavy | 3+ | 3+<br>1. C. | 3+ | 3+<br>1. C. | 3+ | 3+<br>1−5 C<br>1−2 C<br>1−4 C | 3+ | 3+<br>1−6 C<br>1−3 C<br>1−10 C | 3+ | 3− |
| 20 cc. | 3+ | 3+<br>Heavy | 3+ | 3+<br>Heavy | 3+ | 3+<br>Heavy | 3+ | 3+<br>1. C. | 3+ | 3+<br>2−1 C<br>1−2 C | 3+ | 2−1+<br>1 C | 3+ | 2+1−<br>2 C<br>each | 3− | 2−1+<br>1 C |

Cephaeline iso-amyl ether phosphate is germicidal in a dilution of 1:4120 but not in 1:20,200. In the two sets of control tubes, there was no marked difference in the spontaneous death of the organisms. The amount of evaporation in these tubes was considerable, averaging 0.189 cc. per day from the 10 cc. tubes and 0.275 cc. from the 20 cc. tubes.

A third similar test using higher dilutions of cephaeline iso-amyl ether phosphate was run, with 10 cc. of broth and a 0.1 per cent. solution of the salt. Ten dilutions ranging from 1:10,000 to 1:100,000 were tried. The 1:10,000 and 1:11,000 dilutions were inhibitory to the growth of the organism up to three days but were not germicidal.

From these few tests it can only be said that both the propyl and iso-amyl derivatives of cephaeline possess far greater bactericidal action than does emetine. The experiments were too few and too inconclusive to afford an estimate of the degree of this action.

## CONCLUSIONS.

Emetine hydrochloride in solution of 1:1000 when acting on water amebas for one hour, or in solution of 1:5000 acting for three hours, destroyed many of these organisms but was not uniformly amebacidal.

Emetine hydrochloride in solution of 1:200,000 in contact with cultures of amebas for one hour, four hours, or seven hours destroyed many amebas, but transplants from these cultures to fresh agar plates showed a retarded or delayed growth of amebas, due probably to the development of encysted or resistant forms.

Emetine hydrochloride in solutions as strong as 1:100 are not rapidly destructive to the endameba buccalis, in some cases not killing them in one hour.

The propyl and iso-amyl ethers of cephaeline are stronger than emetine as amebacides but their action on water amebas or the Endameba buccalis cannot be used satisfactorily as a comparative measure of this action.

Methylating cephaeline to form emetine is known to increase the endamebacidal action as well as the protozoocidal action toward paramecia and the substitution of the methyl group by ethyl, propyl, butyl, iso-amyl, or allyl further intensifies this action.

The propyl, butyl and iso-amyl ethers of cephaeline possess much stronger protozoocidal properties than the methyl ether (emetine). Cephaeline iso-amyl ether phosphate was the most effective alkaloid of this group in killing paramecia, being fifteen to twenty times as active as emetine phosphate.

Tested on Staphylococcus aureus in the manner described, cephaeline propyl ether phosphate is germicidal in solutions of 1 to 222, and cephaeline iso-amyl ether phosphate in solutions of 1 to 4120. Both of these derivatives are much stronger than emetine in germicidal action.

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# A CONTRIBUTION TO THE PHARMACOLOGY OF COTTON ROOT BARK.

CHARLES R. ECKLER, M. S.

## PURPOSE.

Cotton root bark has been used as a medicine for many years by the negroes of the South, and it is stated that they brought the knowledge of the drug with them from Africa. The drug has been used as a substitute for ergot by some physicians who have claimed that its action resembles that of ergot, and that it has the advantages over the latter of being safer and more stable. This use of cotton root bark has been very largely of an empirical nature, for very little experimental work on animals has been reported and the most of this has seemed unfavorable to the use of the drug. The majority of the clinical reports have also seemed unfavorable. At a comparatively recent date, however, J. C. Scott published a favorable report regarding the action on the cat's uterus. (1) This author states that "gossypii cortex is stable and very active." In the two experiments which he reports, one on the isolated and one on the intact uterus, he seems to have used a powdered extract of the root bark suspended in Ringer's solution, which, in the case of the isolated uterus was applied directly to the organ, and in the case of the intact uterus was apparently given intramuscularly. It became of interest, therefore, to know more about the activity of cotton root bark, as compared with that of ergot in particular, and to some extent with that of pituitary extract. It became of interest, also, to learn whether or not there was any distinct difference in activity between the "green" and the "dried" bark of commerce, and whether or not drug collected at the time of flowering would be more active than the commercial drug which is, in either the case of the "green" or the "dried," collected after the cotton is harvested, and further, whether or not different varieties of the cotton plant would show any distinct difference in activity. The purpose of this work was to gain more definite knowledge regarding these points.

## MATERIAL.

Several commercial samples of "green" and "dried" root bark were tested, and in addition, samples of thirteen different varieties of the cotton plant. The latter were carefully collected at the time of flowering. They were obtained by Mr. F. A. Miller, of our botanical department, from the Georgia Agricultural Experiment Station. Fluidextracts or modified fluidextracts of the drug, only, were tested. Aqueous suspensions of powdered extract were not employed. A part of the commercial "dried" barks were extracted with a menstrum of alcohol and glycerin, 3 to 1, and a part with 75% alcohol. The "green" barks were extracted with the alcohol-glycerin, and the thirteen samples were extracted with 75% alcohol.

Fluidextracts of ergot were used for comparison. These were made from commercial lots of drug, by extraction with 50% alcohol.

The pituitary extracts used in comparative tests were either samples of commercial extract, or laboratory extractions of commercial lots of glands.

## METHODS.

It was decided to test the activity of cotton root barks by the methods commonly employed for the testing of ergot, namely: the cock's comb, blood pressure, and uterus methods. These methods as carried out in this work are briefly as follows:

### COCK'S COMB METHOD.

The cock's comb method consists in administering to purebred, single-comb, white leghorn roosters, by injection into the pectoral muscles, gradually increasing or diminishing doses of fluidextract until a slight but distinct and unmistakable bluing of the comb points has been produced. This method is simple and rapid, and has been reported as giving results which seem to run closely parallel to those obtained by the intact uterus method. Perhaps no other method is so commonly employed for the testing of ergot. The end-point, unlike that of the other methods, is a purely visual one.

### BLOOD PRESSURE METHOD.

The blood pressure method consists in recording the change in carotid pressure caused by the intravenous injection of the fluidextract by way of the saphenous vein. Young, medium sized dogs are the subjects preferred. These have seemed to give the most satisfactory records when anaesthetized with morphine sulphate (.010 to .012 g. per Kg.) and hyoscine hydrobromide (.000,064 to .000,128 g. per Kg.). Ether is used for the operation of inserting cannulae, etc., but is withdrawn as soon as the apparatus is set up and running. Artificial respiration is established in all cases. The common mercury manometer was employed in this work. The doses of fluidextract, calculated per Kg. of body weight, were diluted for injection with one or two times their volume of salt solution.

A few experiments were carried out on decapitated dogs and cats, prepared according to the method of Sherrington (2).

The blood pressure method, essentially as described, is a commonly employed laboratory method for studying the effect of drugs on the circulation. It has been used extensively for determining the pressor effect of preparations of ergot.

### THE ISOLATED UTERUS METHOD.

The isolated uterus method consists in recording the movements of isolated horns of the virgin guinea pig's uterus. These muscular segments are kept bathed in warm oxygenated Locke's solution, and at intervals the drug to be tested is added to the Locke's solution in quantitative manner. The segment being attached to a writing lever, any contractions or relaxations caused by the application of the drug are recorded on a smoked surface. (A more detailed description of the method and apparatus may be found in The Am. Jr. Phar. 89, 195, 1917, and in the Jr. Lab. and Clin. Med. 2, 819, 1917.)

The isolated uterus method is commonly employed for determining the value of pituitary extracts. The method is also used for testing ergot and other drugs which cause, by their application a change in the contractility of involuntary muscle.

### THE INTACT UTERUS METHOD.

The intact uterus method consists in recording the movement of the uterus in situ caused by the intravenous injection of the drug, usually by way of the jugular vein. The animal, anaesthetized with one of the soporific drugs such as acetoform or paraldehyde, is partly immersed in a saline bath of body temperature. The uterus is exposed under the salt solution and attached to a Cushny single myocardiograph, or similar instrument, which records, on a smoked surface, any movements of the organ.

The intact uterus method is a very useful one for studying the qualitative action of those drugs which are used in medicine for their effect on this organ. It seems to be the most logical one for this purpose. As a method of quantitative assay, where many injections have to be made, it is perhaps less serviceable than the isolated uterus method, for the factors governing the uterus are less under control and the organ is influenced to a greater extent by the cumulative action of previous doses of the drug.

### RESULTS OF COCK'S COMB EXPERIMENTS.

The fowls to be used for the testing of cotton root bark were either given, or had been given, doses of ergot. The results of these tests were desired to serve as a control. Fowls vary in their resistance to drugs and occasionally, although rather rarely, one will find combs which do not become distinctly blue after ergot, the effect being more of a blanching. These control tests showed that the combs could be blued by small to medium doses of ergot.

(Table 1, showing the effect of ergot on the combs of test fowls, has been omitted.

A fluidextract of commercial, dried cotton root bark, made with a menstruum of alcohol and glycerin (3 to 1), gave the following results on the fowls previously tested with ergot:

*Table 2*

Cock's Comb Tests on Cotton Root Bark

| Fowl | Weight in Kg. | Dose per Kg. | Results |
|---|---|---|---|
| 394 | 1.545 | 1.0 c.c. | No noticeable bluing of comb |
| 386 | 1.544 | 1.0 c.c. | “ “ “ “ “ |
| 388 | 1.460 | 1.5 c.c. | “ “ “ “ “ |
| 393 | 1.388 | 2.0 c.c. | “ “ “ “ “ |
| 397 | 1.665 | 2.0 c.c. | “ “ “ “ “ |
| 384 | 1.705 | 2.5 c.c. | “ “ “ “ “ |
| 388 | 1.467 | 2.5 c.c. | “ “ “ “ “ |
| 391 | 1.661 | 3.0 c.c. | “ “ “ “ “ |
| 396 | 1.706 | 3.0 c.c. | “ “ “ “ “ |
| 398 | 1.690 | 3.0 c.c. | “ “ “ “ “ |
| 400 | 1.507 | 3.0 c.c. | “ “ “ “ “ |
| 501 | 1.644 | 3.0 c.c. | Very faint bluing of comb |
| 502 | 1.553 | 4.0 c.c. | Extremely faint bluing of comb |
| 503 | 1.532 | 5.0 c.c. | Faint bluing of comb |

A fluidextract of commercial, "green" cotton root bark, made with a menstruum of alcohol and glycerin (3 to 1), gave results as follows:

| Fowl | Weight in Kg. | Dose per Kg. | Results |
|---|---|---|---|
| 511 | 1.703 | 1.0 c.c. | No noticeable bluing of comb |
| 515 | 1.532 | 1.0 c.c. | “ “ “ “ “ |
| 512 | 1.586 | 2.0 c.c. | “ “ “ “ “ |
| 516 | 1.688 | 2.0 c.c. | “ “ “ “ “ |
| 530 | 1.630 | 2.0 c.c. | “ “ “ “ “ |
| 514 | 1.441 | 3.0 c.c. | “ “ “ “ “ |
| 517 | 1.337 | 3.0 c.c. | “ “ “ “ “ |
| 524 | 1.498 | 3.0 c.c. | “ “ “ “ “ |
| 518 | 1.576 | 3.0 c.c. | “ “ “ “ “ |
| 511 | 1.635 | 3.0 c.c. | “ “ “ “ “ |
| 512 | 1.578 | 3.0 c.c. | “ “ “ “ “ |
| 515 | 1.528 | 3.0 c.c. | “ “ “ “ “ |
| 516 | 1.662 | 3.0 c.c. | “ “ “ “ “ |
| 385 | 1.715 | 3.0 c.c. | Extremely faint bluing of comb |

The commercial drug did not, in any case, produce bluing of the cock's comb in doses smaller than 3 c.c. per Kg. The dose of ergot required for these same fowls was from 0.50 to 1 c.c. Other symptoms were evident, however, such as blanching of the comb, wattles, and areas about the eyes, drooping of the feathers, dyspnoea and diarrhoea. With doses of 4 and 5 c.c. per Kg. the fowls seemed very sick, and while slight bluing was produced, the end point was so indefinite that a quantitative assay by this method seemed to be quite impossible. Doses of 2 and 3 c.c. per Kg. of each of the thirteen samples were then given to fowls, the combs of which had previously been blued by small doses of ergot. From the results shown in Table 3 it may be seen that none of the thirteen samples were distinctly active in bluing the comb in doses of 3 c.c. per Kg. This dose is four times the amount which was required of fluidextract of ergot to produce a distinct bluing in the same fowls. Testing by the cock's comb method was therefore discontinued.

*Table 3*
Tests on Samples of Different Varieties of
Cotton Root Bark, Collected at Flowering
*Cock's Comb Method*

| Sample | Fowl | Weight | Dose per Kg. | Results |
|---|---|---|---|---|
| No. 1 | 540 | 2.000 | 2 c.c. | No noticeable bluing of comb. |
| | 544 | 1.713 | 3 c.c. | " " " " " |
| No. 2 | 535 | 1.424 | 2 c.c. | " " " " " (?) (Cold) |
| | 548 | 1.785 | 3 c.c. | " " " " " |
| No. 3 | 545 | 1.921 | 2 c.c. | " " " " " (Redder) |
| | 540 | 1.404 | 3 c.c. | " " " " " |
| No. 4 | 541 | 1.852 | 2 c.c. | " " " " " (Redder) |
| | 541 | 1.923 | 3 c.c. | " " " " " |
| No. 5 | 554 | 1.703 | 2 c.c. | " " " " " (Redder) |
| | 535 | 1.547 | 3 c.c. | " " " " " |
| No. 6 | 548 | 1.908 | 2 c.c. | " " " " " |
| | 545 | 1.924 | 3 c.c. | " " " " " Warm red |
| No. 7 | 540 | 1.957 | 2 c.c. | " " " " " (?) (Cold) |
| | 541 | 1.981 | 3 c.c. | " " " " " (?) (Cold) |
| No. 8 | 541 | 1.859 | 2 c.c. | " " " " " (?) (Cold) |
| | 540 | 2.106 | 3 c.c. | " " " " " (?) (Cold) |
| No. 9 | 545 | 1.871 | 2 c.c. | No noticeable bluing of comb. |
| | 548 | 1.776 | 3 c.c. | " " " " " (?) (Cold) |
| No. 10 | 545 | 1.342 | 2 c.c. | " " " " " |
| | 544 | 1.746 | 3 c.c. | " " " " " (?) (Cold) |
| No. 11 | 535 | 1.342 | 2 c.c. | " " " " " |
| | 535 | 1.584 | 3 c.c. | " " " " " (?) (Cold) |
| No. 12 | 544 | 1.678 | 2. cc. | " " " " " |
| | 539 | 1.716 | 3 c.c. | " " " " " (?) (Cold) |
| No. 13 | 548 | 1.866 | 2 c.c. | " " " " " |
| | 546 | 1.775 | 3 c.c. | Extremely faint bluing of comb (?) |

In the cases marked (?) there seemed to be a mere suggestion of bluing, so faint, however, that it could not be distinguished with certainty. No. 13 with 3 c. c. was the most pronounced of these.

## BLOOD PRESSURE EXPERIMENTS.

Before presenting the results of blood pressure experiments on cotton root bark, results of normal blood pressures of anaesthetized dogs were observed in order to determine with what regularity the normal pressure would run when recorded by the method and apparatus employed. Such results may be seen in Table 4. Slight variations in pressure would probably be recorded with the best of apparatus and under the most careful technic. Occasionally, however, more pronounced changes may be caused by a slight movement of the animal as happened in experiment 786. A somewhat more disturbing change is such as may be seen in experiment 788, where there is for some little time a slight but gradual fall in pressure, and in 779, where there is a slight but gradual rise. In both cases, however, the pressure ran with fair uniformity after the 10 minute period. In experiment 767 a slight rise appears after the 20 minute period. No cause can be assigned for these changes except by supposing that the force or rate of the respiration was not such as to quite accommodate the needs of the particular animals. These points should be considered when viewing Table 6, 6A and 6B, which show the results of blood pressure experiments on cotton root bark. Three of the kymograms may be seen, also, in Chart 1.

*Table 4* *Records of Normal Blood Pressures of Dogs*
(Morphine-Hyoscine Anaesthesia)
Pressure given in millimeters

| | | | Kymograms | | | |
|---|---|---|---|---|---|---|
| Time | 767 Dog 10.2 Kg. | 773 Dog 8.3 Kg. | 788 Dog 11.6 Kg. | 775 Dog 6.2 Kg. | 779 Dog 19.3 Kg. | 786 Dog 10 Kg. |
| Start | 110 | 119 | 148 | 125 | 104 | 123 |
| 5′ | 108 | 120 | 138 | 115 | 117 | 120 |
| 10′ | 110 | 127 | 135 | 106 | 127 | 117 |
| 15′ | 110 | 124 | 132 | 105 | 133 | 114 |
| 20′ | 109 | 125 | 130 | 105 | 133 | 120 |
| 25′ | 116 | 122 | 130 | 105 | 134 | animal |
| 30′ | 116 | 122 | 129 | 106 | 135 | moved |
| 35′ | 119 | 118 | 127 | 119 | 134 | 141 |
| 40′ | 122 | 117 | 126 | | 134 | 139 |
| 45′ | 122 | 118 | 126 | | 134 | 135 |
| 50′ | 122 | 118 | 128 | | 134 | |
| 55′ | 120 | 114 | 127 | | 138 | |
| 60′ | 118 | 116 | | | 138 | |
| 65′ | 115 | 115 | | | 138 | |
| 70′ | 113 | 115 | | | | |
| 75′ | 112 | 118 | | | | |
| 80′ | 109 | | | | | |
| 100′ | 111 | | | | | |
| 115′ | 107 | | | | | |

A few blood pressure tracings were made, showing the effect of ergot, in order to compare the action of cotton root bark with that of ergot on the circulation, and to show how extensively the normal pressure may be influenced. Table 5 shows the results of six ergot blood pressure experiments and gives one a fair idea of the great elevation in pressure which is almost invariably produced by carefully prepared fluidextracts of this drug.

*Table 5*
*Blood Pressure Records on Samples of Fluid Extract of Ergot, Taken on Dogs*

| Dog Wt. in Kg. | Dose per Kg. | Normal Pressure | Pressure at Periods After Injection of Drug | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1–3′ | 5′ | 10′ | 15′ | 20′ | 25′ | 30′ |
| 12.7 | .05 c.c. | 147 | 225 | 218 | 187 | 187 | 185 | 178 | 174 |
| 10.6 | .05 c.c. | 147 | 237 | 226 | 206 | 195 | 189 | 188 | 185 |
| 14.4 | .05 c.c. | 150 | 214 | 214 | 200 | 176 | 167 | (150at42′) | |
| 6.6 | .10 c.c. | 144 | 263 | 220 | 204 | 190 | 188 | 181 | |
| 10.5 | .10 c.c. | 129 | 175 | 198 | 190 | 184 | 182 | 180 | |
| 22.2 | .10 c.c. | 144 | 190 | 172 | 220 | 234 | 220 | 216 | 210 |

*Table 6*

*Blood Pressure Records of Thirteen Samples of Cotton Root Bark on Dogs*

| Drug | Dose per Kg. | Pressure at Start | Pressure at Periods After Injection | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1–3′ | 5′ | 10′ | 15′ | 20′ | 25′ | 20 |
| (Kymogram 736. Dog Wt. 12 Kg. Morphine S.-Hyoscine HBr. anaesthesia.) | | | | | | | | | |
| Cotton R. No. 1 | .1 c.c. | 120 | 124 | 118 | clot | 104 | | | |
| | .2 c.c. | 100 | 116 | 107 | 114 | 114 | | | |
| F. E. Ergot | .05 c.c. | 115 | 153 | 131 | 133 | | | | |
| (Kymogram 772. Dog Wt. 10.5 Kg. Morphine S.-Hyoscine Hbr.) | | | | | | | | | |
| Cotton R. No. 1 | .05 c.c. | 135 | 138 | 135 | 132 | 130 | | | |
| | .1 c.c. | 130 | 142 | clot | 130 | 134 | 134 | | |
| | .2 c.c. | 127 | 145 | 123 | 126 | | | | |
| (Kymogram 773. Dog Wt. 8.3 Kg. Morphine S.-Hyoscine HBr.) | | | | | | | | | |
| Cotton R. No. 1 | .2 c.c. | 117 | 133 | 117 | 114 | | | | |
| F. E. Ergot | 1. c.c. | 114 | 133 | | | | | | |
| (Kymogram 741. Dog Wt. 8.8 Kg. Morphine S.-Hyoscine Hbr.) | | | | | | | | | |
| Cotton R. No. 2 | .1 c.c. | 137 | 147 | 142 | 145 | 152 | 157 | | |
| | .2 c.c. | 157 | 168 | 153 | 153 | clot | | 143 | |
| F. E. Ergot | .05 c.c. | 143 | 158 | 179 | 164 | 162 | | | |
| (Kmogram 778. Dog Wt. 11.6 Kg. Morphine S.Hyoscine HBr.) | | | | | | | | | |
| Cotton R. No. 2 | .2 c.c. | 127 | 137 | 130 | 126 | 124 | | | |
| F. E. Ergot | .05 c.c. | 124 | 140 | 140 | 140 | | | | |
| | .05 c.c. | 140 | 155 | 148 | 154 | | | | |
| (Kymogram 778-A. Dog Wt. 11.7 Kg. Morphine S-.Hyoscine HBr.) | | | | | | | | | |
| Cotton R. No. 3 | .1 c.c. | 105 | 117 | 108 | 108 | 104 | 103 | | |
| | .2 c.c. | 103 | 122 | 106 | clot | 127 | 119 | | |
| F. E. Ergot | .05 c.c. | 122 | 149 | 134 | 129 | | | | |
| (Kymogram 743. Dog Wt. 8 Kg. Morphine S.-Hyoscine HBr.) | | | | | | | | | |
| Cotton R. No. 3 | .2 c.c. | 126 | 135 | 118 | 118 | 119 | 113 | | |
| | .3 c.c. | 113 | 120 | 105 | 108 | 109 | 112 | | |
| F. E. Ergot | .05 c.c. | 113 | 134 | 129 | 131 | 137 | 133 | 133 | |
| | .1 c.c. | 133 | 160 | 144 | | | | | |
| (Kymogram 779. Dog Wt. 19.3 Kg. Morphine S.-Hyoscine HBr.) | | | | | | | | | |
| Cotton R. No. 3 | .1 c.c. | 138 | 152 | 138 | 140 | 140 | | | |
| (Kymogram 749. Dog Wt. 10 Kg. Morphine S.-Hyoscine HBr.) | | | | | | | | | |
| Cotton R. No. 4 | .1 c.c. | 113 | 128 | 121 | 125 | 124 | 126 | 124 | |
| | .2 c.c. | 124 | 146 | 123 | 131 | 133 | | | |
| (Kymogram 750. Dog Wt. 9.4 Kg. Morphine S.-Hyoscine HBr.) | | | | | | | | | |
| Cotton R. No. 5 | .1 c.c. | 125 | 144 | 135 | 132 | 130 | 130 | 130 | |
| | .2 c.c. | 130 | 148 | 143 | 136 | 133 | 134 | | |
| F. E. Ergot | .05 c.c. | 141 | 198 | 195 | 166 | 166 | 150 | 153 | |
| (Kymogram 751. Dog Wt. 5.9 Kg. Morphine S.-Hyoscine HBr.) | | | | | | | | | |
| Cotton R. No. 6 | .1 c.c. | 149 | 152 | 144 | 150 | 155 | 154 | 154 | |
| | .2 c.c. | 154 | 176 | 154 | 158 | clot | | 141 | |
| F. E. Ergot | .05 c.c. | 130 | 176 | 151 | 154 | 150 | 141 | 136 | |
| (Kymogram 753. Dog Wt. 8.8 Kg. Morphine S.-Hyoscine HBr.) | | | | | | | | | |
| Cotton R. No. 7 | .1 c.c. | 142 | 148 | 146 | 154 | 155 | 155 | | |
| | .2 c.c. | 158 | 164 | 161 | 157 | 152 | 147 | 144 | |
| F. E. Ergot | .05 c.c. | 145 | 168 | 169 | 160 | 158 | 157 | | |
| (Kymogram 756. Dog Wt. 13.6 Kg. Morphine S.-Hyoscine HBr.) | | | | | | | | | |
| Cotton R. No. 8 | .1 c.c. | 121 | 137 | 125 | 122 | 118 | 120 | 122 | |
| | .2 c.c. | 139 | 153 | 136 | 138 | 135 | 132 | 131 | |
| F. E. Ergot | .05 c.c. | 131 | 164 | 188 | 187 | 172 | 166 | 155 | |
| (Kymogram 755. Dog Wt. 14 Kg. Morphine S.-Hyoscine HBr.) | | | | | | | | | |
| Cotton R. No. 9 | .1 c.c. | 124 | 139 | 135 | 136 | 143 | 152 | 153 | |
| | .2 c.c. | 150 | 170 | 150 | 140 | 142 | 139 | 138 | |
| F. E. Ergot | .05 c.c. | 130 | 157 | 137 | 130 | 129 | 130 | 132 | |

*Table 6*

*Blood Pressure Records of Thirteen Samples of Cotton Root Bark on Dogs*

| Drug | Dose per Kg. | Pressure at Start | Pressure at Periods After Injection 1–3′ | 5′ | 10′ | 15′ | 20′ | 25′ | 30 |
|---|---|---|---|---|---|---|---|---|---|
| (Kymogram 759. | Dog Wt. 5.5 Kg. | Morphine S.-Hyoscine HBr.) | | | | | | | |
| Cotton R. No. 10 | .1 c.c. | 134 | 143 | 130 | 132 | 129 | 126 | 120 | |
| | .2 c.c. | 120 | 136 | 128 | 127 | 131 | 129 | 127 | |
| F. E. Ergot | .05 c.c. | 127 | 158 | 145 | 148 | 145 | 154 | 157 | |
| (Kymogram 763. | Dog Wt. 11.8 Kg. | Morphine S.-Hyoscine HBr.) | | | | | | | |
| Cotton R. No. 11 | .1 c.c. | 114 | 125 | 120 | 117 | 123 | 120 | 123 | |
| | .2 c.c. | 123 | 132 | 124 | 121 | 118 | 118 | 122 | |
| F. E. Ergot | .05 c.c. | 122 | 140 | 138 | 136 | | | | |
| | .1 c.c. | 136 | 157 | 133 | 124 | 121 | | | |
| (Kymogram 764. | Dog Wt. 11.2 Kg. | Morphine S.-Hyoscine HBr.) | | | | | | | |
| Cotton R. No. 12 | .1 c.c. | 112 | 123 | 135 | 120 | 120 | 120 | 117 | |
| | .2 c.c. | 117 | 127 | 123 | 126 | 117 | 117 | | |
| F. E. Ergot | .05 c.c. | 118 | 172 | 170 | 169 | 152 | 140 | | |
| | .1 c.c. | 127 | 147 | 137 | 142 | | | | |
| (Kymogram 765. | Dog Wt. 8.9 Kg. | Morphine S.-Hyoscine HBr.) | | | | | | | |
| Cotton R. No. 13 | .1 c.c. | 116 | 124 | 129 | 128 | 133 | 133 | | |
| | .2 c.c. | 133 | 147 | 141 | 137 | 137 | 133 | | |
| F. E. Ergot | .05 c.c. | 116 | 140 | 140 | | | | | |

*Table 6-A*

*Blood Pressure Records of Commercial Cotton Root Bark on Dogs*

| Drug | Dose per Kg. | Pressure at Start | Pressure at Periods After Injection 1–3′ | 5′ | 10′ | 15′ | 20′ | 25′ |
|---|---|---|---|---|---|---|---|---|
| (Kymogram 902. | Dog Wt. 4.7 Kg. | Morphine Sulphate Anesathesia.) | | | | | | |
| F. E. C. (Dry) | .1 c.c. | 144 | 160 | 145 | 143 | 134 | | |
| 40′ later = | .1 c.c. | 106 | 126 | 118 | 117 | | | |
| (Kymogram 901. | Dog Wt. 10 Kg. | Morphine Sulphate Anaesthesia.) | | | | | | |
| F. E. C. (Dry) | .1 c.c. | 110 | 138 | 136 | 127 | 120 | 120 | |
| | .15 c.c. | 120 | 147 | 134 | 136 | 136 | 136 | |
| | .20 c.c. | 136 | 138 | | | 122 | 134 | 131 |
| (Kymogram 900. | Dog Wt. 11.8 Kg. | Morphine Sulphate Anaesthesia.) | | | | | | |
| Alcohol-glyc. | .15 c.c. | 136 | 144 | | | | | |
| (3 to 1) | .15 c.c. | 136 | 136 | | | | | |
| F. E. C. (Dry) | .15 c.c. | 143 | 159 | 145 | 145 | | | |
| | .20 c.c. | 145 | 158 | 122 | 141 | 140 | | |
| F. E. Ergot | .15 c.c. | 140 | 178 | 170 | 158 | 158 | 151 | 149 |
| F. E. C. (Dry) | .15 c.c. | Caused death | | | | | | |
| (Kymogram 894. | Dog Wt. 12 Kg. | Morphine Sulphate Anaesthesia.) | | | | | | |
| Alcohol-glyc. | .2 c.c. | 111 | 119 | | | | | |
| F. E. C. (Dry) | .2 c.c. | 118 | 128 | 119 | 120 | 114 | | |
| F. E. Ergot | .2 c.c. | 123 | 114 | clot | 124 | | | |
| (Kymogram 893. | Dog Wt. 10 Kg. | Morphine Sulphate Anaesthesia.) | | | | | | |
| Adrenal Ext. | | 116 | 146 | 130 | | | | |
| F. E. C. (Dry) | .1 c.c. | 129 | 138 | 114 | 110 | | | |
| | .15 c.c. | 110 | 115 | clot | 112 | 95 | | |
| | .2 c.c. | 103 | 110 | 101 | 101 | | | |
| | .3 c.c. | 101 | Fall | Slow recovery. | | | | |
| (Kymogram 891. | Dog Wt. 7 Kg. | Morphine Sulphate Anaesthesia.) | | | | | | |
| Adrenal Ext. | | 135 | 157 | 143 | 143 | | | |
| F. E. C. (Dry) | .2 c.c. | 143 | 164 | 143 | 140 | | | |
| | .2 c.c. | 139 | 150 | 120 | 117 | 123 | 126 | |
| | .2 c.c. | 126 | 136 | 124 | 127 | 127 | | |
| | .3 c.c. | Gradual fall for 5′ then slow recovery. | | | | | | |
| F. E. Ergot | .15 c.c. | Fall, slight rise, then gradual lowering. | | | | | | |

*Table 6-A*

*Blood Pressure Records*

*of Commercial Cotton Root Bark on Dogs*

| Drug | Dose per Kg. | Pressure at Start | Pressure at Periods After Injection | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1–3′ | 5′ | 10′ | 15′ | 20′ | 25′ |
| (Kymogram 890. | Dog Wt. 8.6 Kg. | Morphine Sulphate Anasethesia.) | | | | | | |
| Adrenal Ext. | | 143 | 170 | 140 | | | | |
| Alcohol-glyc. | .3 c.c. | No effect | | | | | | |
| F. E. C. (Dry) | .2 c.c. | 146 | 155 | 128 | 126 | clot | 132 | 122 |
| | .3 c.c. | 122 | 140 | 84 | 93 | clot | 102 | 107 |
| F. E. Ergot | .15 c.c. | Slight rise, then gradual fall. | | | | | | |
| (Kymogram 691. | Dog Wt. 10.8 Kg. | Morph. S.-Hyoscine HBr.) | | | | | | |
| After Ergot. | | | | | | | | |
| F. E. C. (Dry) | .05 c.c. | 125 | 135 | 130 | 128 | 125 | 123 | 122 |
| (Kymogram 892. | Dog Wt. 27.4 Kg. | Morph. S.-Atropine S.) | | | | | | |
| F. E. C. (Green) | .2 c.c. | 143 | 154 | 146 | 146 | 142 | 144 | |
| F. E. Ergot | .1 c.c. | 144 | 210 | 210 | 204 | 200 | 202 | |
| (Kymogram 982. | Dog Wt. 11 Kg. | Morph. S.-Hyoscine HBr. Cut Vagi.) | | | | | | |
| F. E. C. (Green) | .2 c.c. | 122 | 144 | 126 | 127 | | | |
| Alc.-glycerin | .2 c.c. | No effect | | | | | | |
| F. E. Ergot | .05 c.c. | 132 | 190 | 163 | 156 | 135 | 130 | |
| F. E. C. (Green) | .2 c.c. | No effect | | | | | | |
| (Kymogram 895. | Dog Wt. 2.7 Kg. | Morph. S.-Atropine S.) | | | | | | |
| F. E. C. (Green) | .2 c.c. | 112 | 134 | 132 | 132 | 134 | 134 | 130 |
| | .2 c.c. | 130 | 148 | 124 | 124 | 118 | 112 | 112 |
| F. E. Ergot | .1 c.c. | 112 | 154 | 138 | 131 | 120 | 119 | 119 |

*Table 6-B*

*Blood Pressure Records*

*of Commercial Cotton Root Bark on*

*Spinal Preparations*

| Drug | Dose per Kg. | Pressure at Start | Pressure at Periods After Injection | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1–3′ | 5′ | 10′ | 15′ | 20′ | 25′ |
| (Kymogram 896. | Preparation Wt. 2.2 Kg. | Cat.) | | | | | | |
| F. E. C. (Dry) | .2 c.c. | 82 | 46–69 | 62 | 67 | | | |
| F. E. Ergot | .2 c.c. | 17 | 38–138 | 134 | 126 | 110 | 100 | 78 |
| F. E. C. | .2 c.c. | Abrupt fall. Heart stopped. Epinephrin was given and the heart massaged. Heart began beating. Later ergot caused a rise in pressure. | | | | | | |
| (Kymogram 898. | Preparation Wt. 2.4 Kg. | Cat.) | | | | | | |
| Alc.-glyc. (3 to 1) | .16 c.c. | 58 | 60 | 56 | | | | |
| F. E. C. (Dry) | .16 c.c. | 58 | 48 | 46 | | | | |
| F. E. Ergot | .08 c.c. | 44 | grad. rise | 80 | 75 | 66 | | |
| F. E. C. | .16 c.c. | 64 | Fall. Heart stopped. Massaging and epinephrin started heart. | | | | | |
| F. E. C. | .08 c.c. | Gradual fall. | | | | | | |
| F. E. C. | .08 c.c. | Pressure fell nearly to zero, heart stopped but recovered after massaging and epinephrin. | | | | | | |
| (Kymogram 4015. | Dog preparation Wt. 7.4 Kg.) | | | | | | | |
| F. E. C. (Dry) | .10 c.c. | 65 | 70–63 | 56 | 51 | | | |
| F. E. Ergot | .05 c.c. | 51 | 65–62 | 66 | 65 | 61 | 60 | |
| F. E. C. | .10 c.c. | 60 | 62–59 | 56 | | | | |
| F. E. Ergot | .10 c.c. | 55 | 63–58 | 60 | | | | |
| Pit. Ext. 1:1000 | .20 c.c. | 60 | 63–67 | | | | | |

## COTTON ROOT BARK BLOOD PRESSURE EXPERIMENTO ON THE CAT.

Four blood-pressure experiments were carried out on cats. These animals were anaesthetized with acetoform. In one case .2 c.c. per Kg. of F. E. Cotton R. B. caused almost no effect. In the other three cases .1 and .2 c.c. doses per Kg. caused an abrupt and extensive fall, the pressure slowly regaining the normal height or slightly above the normal. Following is an example:

| | |
|---|---|
| Pressure at start | 86 mm. |
| .13 c.c. per Kg. F. E. C. injected. | |
| Pressure 1-3′ after injection | 27 mm. |
| Pressure 5′ after injection | 92 mm. |
| Pressure 7′ after injection | 86 mm. |

The foregoing work was completed about June 1, 1914. The following experiments were carried out during the spring of 1919.

## ISOLATED UTERUS EXPERIMENTS.

For the uterus experiments, fresh samples of commercial drug were extracted and made into fluidextracts by the N. F. method.

The isolated uterus is, in most cases, an extremely sensitive organ, easily influenced by the chemical nature of the solutions applied to it. Any considerable amount of acid, alcohol, or astringent principle in the solution applied is likely to impair the results of the experiment. Any substance to be applied to the structure should, therefore, be in a clear, neutral or nearly neutral, saline solution. Such a solution fully representing the fluidextract of cotton root bark seemed impossible, for any extensive change in the menstruum, such as would necessarily occur when the fluid was diluted in the Locke's solution surrounding the uterus, caused a precipitation of considerable extractive matter. Such a mixture, however, with its very fine, fresh precipitate, was considered preferable to a suspension of the powdered extract. The procedure employed was to dilute the fluidextract with 9 parts of saline solution, and of this 10% mixture, the doses for application were drawn off. These doses were further diluted from 50 to 100 times when introduced into the Locke's solution surrounding the uterus. The effect of any astringent matter in this dilute solution was disregarded. It would seem to be very slight if, in truth, any were exerted. According to the work of Power and Browning, cotton root bark contains no tannin, (3). The effect of the alcohol, however, was studied by applying percentages equal to, and greater than that found in the solutions of cotton root bark.

Table 7 shows the results of fifteen experiments on the isolated guinea pig's uterus.

### *Table 7*

### ISOLATED UTERUS EXPERIMENTS

*Guinea Pig's Uterus*
*Commercial Drug*

| Experiment or Kymo. | Drug | Dilution | Effect | | (Contractions ,etc.) |
|---|---|---|---|---|---|
| 1 | Pituitary Extract | 1:300,000 | 85 | m.m | contraction |
| | Alcohol | 1: 1,333 | 21 | “ | “ |
| | “ | 1: 1,333 | 17 | “ | “ |
| | Pituitary Extract | 1:300,000 | 67 | “ | “ |
| 2 | Pituitary Extract | 1:300,000 | 87 | “ | “ |
| | Alcohol | 1: 1,333 | 53 | “ | “ |
| | “ | 1: 1,333 | 45 | “ | “ |
| | Pituitary Extract | 1:300,000 | 92 | “ | “ |
| 3E* | Alcohol | 1: 1,333 | 19 | m.m. | contraction |
| | F. E. Cotton R. B. (N. F.) | 1: 1,000 | 38 | “ | “ |
| | Alcohol | 1: 1,333 | 20 | “ | “ |
| | F. E. Cotton R. B. (N. F.) | 1: 1,000 | 48 | “ | “ |
| | Alcohol | 1: 1,000 | 22 | “ | “ |
| 4 | Pituitary Extract | 1:250,000 | 27 | mm. | contraction |
| | Alcohol-glycerine, 3–1 | 1: 100 | Relaxation (slight) | | |
| | “ “ | 1: 1,000 | No noticeable effect | | |
| | F. E. Cotton R. B. (N. F.) | 1: 1,000 | “ | “ | “ |
| | F. E. Ergot | 1: 5,000 | 5 | mm. | contraction |
| 5 | Pituitary Extract | 1:300,000 | 26 | mm. | contraction |
| | Alcohol-glycerin, 3–1 | 1: 1,000 | No noticeable effect | | |
| | F. E. Cotton R. B. (N. F.) | 1: 1,000 | 2 | mm. | contraction |
| | F. E. Ergot | 1: 5,000 | 4 | “ | “ |
| | F. E. Cotton R. B. (N. F.) | 1: 1,000 | No noticeable effect | | |
| | F. E. Ergot | 1: 5,000 | 3.5 | mm. | contraction |
| 6D | Pituitary Extract | 1:250,000 | 24 | mm. | contraction |
| | “ “ | 1:250,000 | 21 | “ | “ |
| | F. E. Cotton R. B. (N. F.) | 1: 1,000 | 1.5 | “ | “ |
| | F. E. Ergot | 1: 4,000 | 24 | “ | “ |
| 7C | Pituitary Extract | 1:250,000 | 87 | mm. | contraction |
| | F. E. Cotton R. B. (N. F.) | 1: 1,000 | 14 | “ | “ |
| | F. E. Ergot | 1: 10,000 | 70 | “ | “ |
| 8B | Pituitary Extract | 1:200,000 | 33 | mm. | contraction |
| | F. E. Cotton R. B. (N. F.) | 1: 500 | 9 | “ | “ |
| | F. E. Ergot | 1: 5,000 | 24 | “ | “ |
| 9 | Pituitary Extract | 1:300,000 | 107 | mm. | contraction |
| | F. E. Cotton R. B. (N. F.) | 1: 500 | 55 | “ | “ |
| 10G | F. E. Ergot | 1: 10,000 | 48 | mm. | contraction |
| | F. E. Cotton R. B. (N. F.) | 1: 1,000 | 26 | “ | “ |
| | F. E. Ergot | 1: 10,000 | 43 | “ | “ |
| 11 | Pituitary Extract | 1:300,000 | 122 | mm. | contraction |
| | F. E. Cotton R. B. (N. F.) | 1: 133 | 115 | “ | “ |
| | Pituitary Extract | 1:300,000 | 123 | “ | “ |
| 12F | Pituitary Extract | 1:250,000 | 30 | mm. | contraction |
| | F. E. Cotton R. B. (N. F.) | 1: 1,000 | 6 | “ | “ |
| | Pituitary Extract | 1:250,000 | 11 | “ | “ |
| | F. E. Ergot | 1: 10,000 | 21 | “ | “ |
| | Pituitary Extract | 1:250,000 | 73 | “ | “ |
| | F. E. Cotton R. B. (N. F.) | 1: 1,000 | 6 | “ | “ |
| | “ | 1: 1,000 | 4 | “ | “ |
| | F. E. Ergot | 1: 10,000 | 5 | “ | “ |
| | “ | 1: 10,000 | 5 | “ | “ |
| | “ | 1: 10,000 | 10 | “ | “ |
| | “ | 1: 10,000 | 31 | “ | “ |
| | F. E. Cotton R. B. (N. F.) | 1: 1,000 | 22 | “ | “ |

*Table 7*

Isolated Uterus Experiments

*Guinea Pig's Uterus*

*Commercial Drug*

| Experiment or Kymo. | Drug | Dilution | Effect | (Contractions, etc.) | |
|---|---|---|---|---|---|
| 13 | Pituitary Extract | 1:300,000 | 52 mm. | contraction | |
| | Alcohol | 1: 1,333 | 16 “ | “ | |
| | “ | 1: 1,000 | 13 “ | “ | †H.T. |
| | “ | 1: 1,333 | 10 “ | “ | H.T. |
| | Pituitary Extract | 1:300,000 | 42 “ | “ | H.T. |
| | F. E. Cotton R. B. (N. F.) | 1: 1,000 | 14 “ | “ | L.T. |
| | “ | 1: 1,000 | 13 “ | “ | L.T. |
| | “ | 1: 1,000 | 4 “ | “ | L.T. |
| | Pituitary Extract | 1:300,000 | 6 “ | “ | L.T. |
| | “ “ | 1:300,000 | 7 “ | “ | L.T. |
| 14 | Pituitary Extract | 1:250,000 | 14 mm. | contraction | |
| | “ “ | 1:250,000 | 13.5 “ | “ | |
| | Alcohol | 1: 1,333 | 0 “ | “ | |
| | “ | 1: 1,333 | 1 “ | “ | |
| | “ | 1: 1,333 | 1 “ | “ | |
| | “ | 1: 1.333 | 1 (?) “ | “ | |
| | Pituitary Extract | 1:250,000 | 30 “ | “ | |
| | “ “ | 1:240,000 | 32 “ | “ | |
| | F. E. Cotton R. B. (N. F.) | 1: 1,000 | 5 “ | “ | |
| | “ | 1: 1,000 | 4 “ | “ | |
| | “ | 1: 1,000 | 2 “ | “ | |
| | Pituitary Extract | 1:250,000 | 2.5 “ | “ | |
| | “ “ | 1:250,000 | 4.5 “ | “ | |
| 15A | Pituitary Extract | 1:250,000 | 65 mm. | contraction | |
| | “ “ | 1:250,000 | 66 “ | “ | |
| | F. E. Cotton R. B. (N. F.) | 1: 1,000 | 9 “ | “ | |
| | Pituitary Extract | 1:300,000 | 42.4 “ | “ | |
| | F. E. Ergot | 1: 5,000 | 70 “ | “ | |
| | “ | 1: 10,000 | 64 “ | “ | |
| | F. E. Cotton R. B. (N. F.) | 1: 1,000 | 62 “ | “ | |
| | “ | 1: 1,000 | 40 “ | “ | |
| | “ | 1: 1,000 | 10 “ | “ | |
| | F. E. Ergot | 1: 10,000 | 17.5 “ | “ | |
| | “ | 1: 10,000 | 38.5 “ | “ | |
| | F. E. Cotton R. B. (N. F.) | 1: 1,000 | 30 “ | “ | |

---

†H. T. = Higher Tonus. L. T. = Lower Tonus.

*The letters indicate the corresponding kymograms on Chart 2.

## INTACT UTERUS EXPERIMENTS.

The intact uterus experiments were carried out on both cats and rabbits. Nothing was known regarding the previous condition of the cats. For the experiments, they were anaesthetized with acetoform. The rabbits were anaesthetized with paraldehyde. Those which had had young, had previously been used for certain biological tests. The virgins were young animals which were raised at the laboratory. Tables 8 and 9 give the results recorded in these experiments.

*Table 8* Intact Uterus Experiments—Cats

Kymogram 904. Cat Weight 2.38 Kg. Acetoform anaesthesia.
.5 c.c. per animal F. E. Cotton R. B. = No contraction.
.5 c.c. “ “ F. E. Ergot = Marked contraction.
.5 c.c. “ “ F. E. Cotton R. B. = No contraction.

Kymogram 905. Cat Weight 2.3 Kg. Acetoform anaesthesia.
.2 c.c. per Kg. F. E. Cotton R. B. = Decided increase in tonus.
.2 c.c. “ “ “ = Slight “ “ “
.2 c.c. “ “ “ = Hardly noticeable effect.
.2 c.c. “ “ F. E. Ergot = Slight increase in tonus.
.2 c.c. “ “ “ = “ “ “ “
.2 c.c. “ “ F. E. Cotton R. B. = Very slight increase in tonus.
.2 c.c. “ “ F. E. Ergot = Slight increase in tonus.
.2 c.c. “ “ Alc.-glyc. (3–1) = No effect.
.4 c.c. “ “ Pituitary Ext. = Slight increase in tonus.

Kymogram 903. Cat Weight 2.8 Kg. Morph. S.-acetoform anaesthesia.
.5 c.c. per animal F. E. Ergot = No contraction.
.005 g. Ergotoxine Phosphate = Increased tonus.
1 c.c. Pituitary Extract = “ “
1 c.c. F. E. Cotton R. B. = No effects.

Kymogram 909. Cat Weight 3.05 Kg. Morph. S.-acetoform anaesthesia.
.5 c.c. per animal F. E. Ergot = No contraction of uterus.
.5 c.c. “ “ “ = Distinct contraction.
.5 c.c. “ “ “ (While uterus was slightly contracted.)
= Relaxation and then contraction.
.5 c.c. “ “ F. E. C. R. B. = Relaxation.
.2 c.c. “ “ Pituitary Ext. = Distinct contraction.

Kymogram 907. Cat Weight 2.6 Kg. Morph. S.-acetoform anaesthesia.
.5 c.c. per animal F. E. Ergot = Marked contraction of uterus.
.5 c.c. “ “ F. E. C. R. B. = “ “ “ “
.5 c.c. “ “ “ = “ “ “ “
.5 c.c. “ “ “ = “ “ “ “

*Table 9* Intact Uterius Experiments—Rabbits

Kymogram M-3987-A. Weight of rabbit 2.2 Kg. Pregnant. Paraldehyde.
.2 c.c. p. Kg. F. E. C. R. B. (50% Alc.) 35 mm. contraction of uterus.
.2 c.c. p. Kg. F. E. C. R. B. (75% Alc.) 28 mm. “ “ “
.05 c.c. p. Kg. F. E. Ergot (50% Alc.) 50 mm. “ “ “

Kymogram 3987. Weight 2 Kg. Non-pregnant, multiparous. Paraldehyde.
.2 c.c. p. Kg. F. E. C. R. B. (50% Alc.) No effect.
.4 c.c. p. Kg. F. E. C. R. B. (75% Alc.) No effect.
.1 c.c. p. Kg. F. E. Ergot (50% Alc.) 4 mm. contraction.

Kymogram 3989. Weight 2.2 Kg. Non-preganant, multiparous. Paraldehyde.
.4 c.c. p. Kg. F. E. C. R. B. (75% Alc.) 12 mm. contraction.
.2 c.c. p. Kg. F. E. Ergot (50% Alc.) 11 mm. “
.2 c.c. p. Kg. 1:1,000 Pituitary Ext. 12 mm. “

Kymogram 3990. Weight 2.3 Kg. Non-pregnant, multiparous. Paraldehyde.
.4 c.c. p. Kg. F. E. C. R. B. (75% Alc.) No effect.
.2 c.c. p. Kg. F. E. Ergot (50% Alc.) 7 mm. contraction.

Kymogram 4204. Weight 1.56 Kg. Virgin. Paraldehyde.
.2 c.c. p. Kg. F. E. Ergot (50% Alc.) Many contr's. One = 47 mm.
.4 c.c. p. Kg. F. E. C. R. B. (75% Alc.) Wave-like contr's. One = 2.5 mm.
.8 c.c. p. Kg. “ “ “ “ “ One = 1 mm.
.2 c.c. p. Kg. F. E. Ergot (50% Alc.) Series of contr's. One = 37.5 mm.

Kymogram 4205. Weight 1.66 Kg. Virgin. Paraldehyde.
.4 c.c. p. Kg. F. E. C. R. B. (75% Alc.) Tone increased 11 mm.
Increased contractions.
.6 c.c. p. Kg. “ “ “ Wave-like contractions = 5 mm.
.2 c.c. p. Kg. F. E. Ergot (50% Alc.) Series of contr's. One = 18.5 mm.
.1 c.c. p. Kg. “ “ “ “ “ “ One = 23.5 mm.

## SUMMARY AND CONCULSIONS.

*Cock's Comb Method.*

Cotton root bark in large doses produced very slight bluing of the cock's comb in a few cases. Doses of the fluidextract fully four times as large as those required of fluidextract of ergot to produce a distinct bluing, failed to produce a comparable effect. Other systemic effects such as dyspnoea and diarrhoea were very pronounced. Owing to these severe symptoms produced by the cotton root bark in the necessarily large doses, and to the unsatisfactory effect on the comb, a close comparison could not be drawn between the activity of this drug and ergot.

By this method, no decided difference could be detected between any of the samples tested, which included root bark from thirteen different varieties of the cotton plant collected at flowering, and several samples of commercial drug both "green" and "dried."

*Blood Pressure Method.*

Cotton root bark produced a slight but transient pressor effect on the blood pressure of the dog. The extent of the effect and the duration were not comparable to that produced by very much smaller doses of ergot. The effect of the cotton root bark usually passed off in 5 or 10 minutes, and occasionally this period was followed by one of lowered pressure. Succeeding doses produced less and less effect. Similarly, ergot following one or more doses of cotton root bark, even though the pressure had not been more than slightly elevated, seemingly did not cause its usual effect.

Five kymograms taken on the intact cat showed either a fall or no effect on the pressure.

On the cat spinal preparation, cotton root bark caused a fall in pressure. One record on a dog spinal preparation showed a slight but very brief rise followed by a fall below normal.

On these preparations, ergot produced a fairly well maintained rise.

By this method, no appreciable or striking difference was noticeable between any of the cotton root products tested.

*Isolated Uterus Method.*

Fluidextract of cotton root bark in rather large doses produced contractions of the isolated guinea pig uterus. A very conservative interpretation of the results obtained would seem to be that cotton root bark possesses decidedly less than 1/10 as much activity as ergot, and decidedly less than 1/300 as much activity as commercial pituitary extract. In fact, when given in doses corresponding to these figures, the contractions produced by the cotton root bark were not comparable to those produced by the other substances. Successive doses of cotton root bark produced smaller and smaller contractions. Similarly, ergot and pituitary extract seemingly did not exert their usual effect after one or more doses of cotton root bark. The opposite effect was often observed when any of the three substances was given after ergot or pituitary extract.

By this method, only commercial samples of cotton root bark were tested. No decided difference could be noted between any of these from the results obtained.

Alcohol in a dilution of 1:1333, approximately the percentage possessed by a 1:1000 dilution of fluidextract of cotton root bark which in several instances was found to be active, caused contractions of the isolated uterus. A 1:100 dilution of alcohol caused, in one instance, an inhibition.

*Intact Uterus Method.*

The results obtained on the intact uterus show that cotton root bark possesses a slight action on the uterus of both the cat and the rabbit. The results on the virgin rabbit's uterus were the most satisfactory. These results indicate that the action of cotton root bark is not comparable to that possessed by ergot. As under the other methods, successive doses of cotton root bark seem to produce less and less effect, and ergot given after cotton root bark seems to produce less than its usual effect.

By this method only commercial samples of cotton root bark were tested. No decided difference could be noted between any of these from the results obtained.

## RECAPITULATION.

Samples of the root bark from thirteen different varieties of the cotton plant, collected at flowering, and a number of samples of commercial root bark, both "green" and "dried," were tested for activity by methods commonly employed for the assay of ergot, namely: the cock's comb, blood pressure, and uterus methods.

Large doses produced a very slight bluing of the cock's comb in a few cases. In general the results by this method were rather indefinite and unsatisfactory, and no decided difference could be detected between any of the samples tested.

A very slight and transient pressor effect was produced on the blood pressure of the dog. On the intact cast a depressor effect or no effect at all was observed. On the cat spinal preparation a depressor effect was observed, and on a dog spinal preparation a pressor followed by depressor.

By this method no decided difference could be noticed between any of the samples tested.

Rather large doses produced small contractions of the isolated guinea pig uterus and the uterus of the cat and rabbit in situ. By the uterus methods only samples of commercial drug were tested. No decided difference could be noticed between any of these from the results obtained.

By the four methods employed, the activity shown by cotton root bark was not comparable to that possessed by ergot.

Tables of results and charts of tracings are given.

I wish to express my obligations to Dr. A. L. Walters, under whose supervision this work was carried out.

Credit is due Mr. C. E. Lawson, for preparing the thirteen experimental samples, and Mr. C. C. Hargreaves and Mr. E. E. Swanson for assistance in carrying out the experiments.

## REFERENCES.

(1) Scott, Therapeutic Gazette, Volume 35, p. 162, 1911.
(2) Sherrington, Journal Physiology, Volume 38, p. 375.
(3) Power and Browning, Pharmaceutical Journal, Volume 93, p. 420-423.

## CHART 1.

Kymogram 1294 shows the effect of fluidextract of ergot on the blood pressure of the dog.

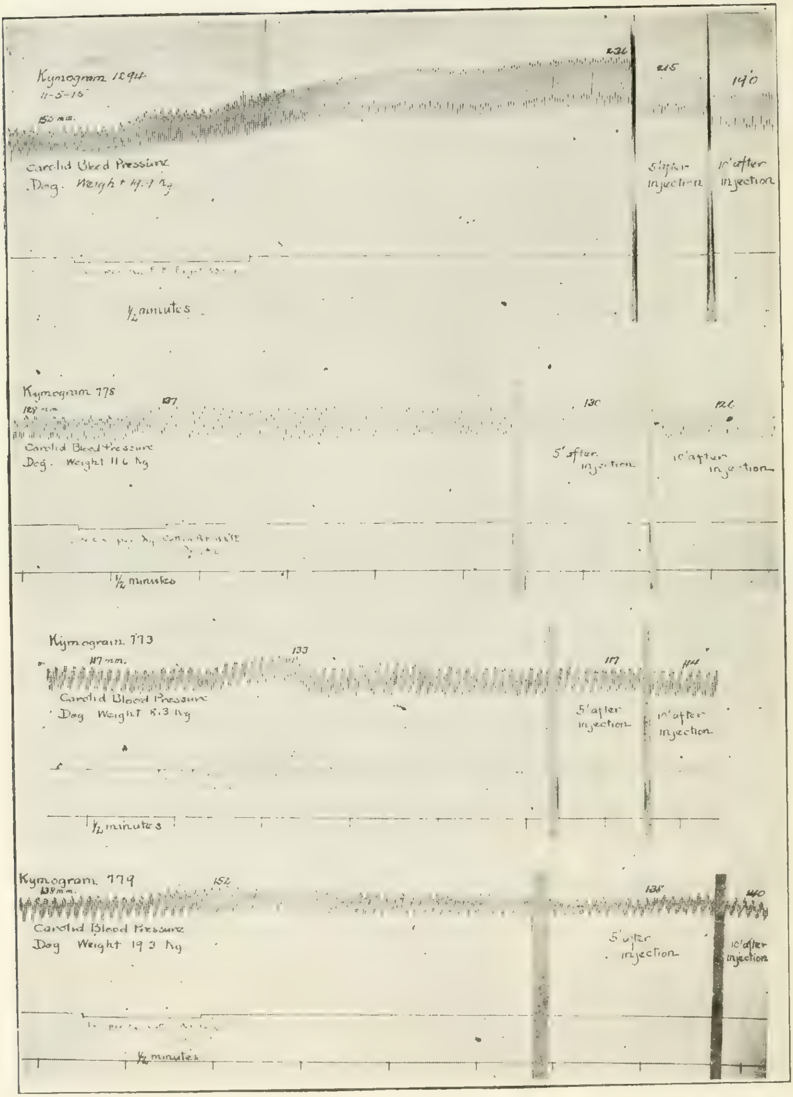
Kymograms 778, 773 and 779 represent three different blood pressure experiments which show the effect of cotton root bark on the blood pressure of the dog.

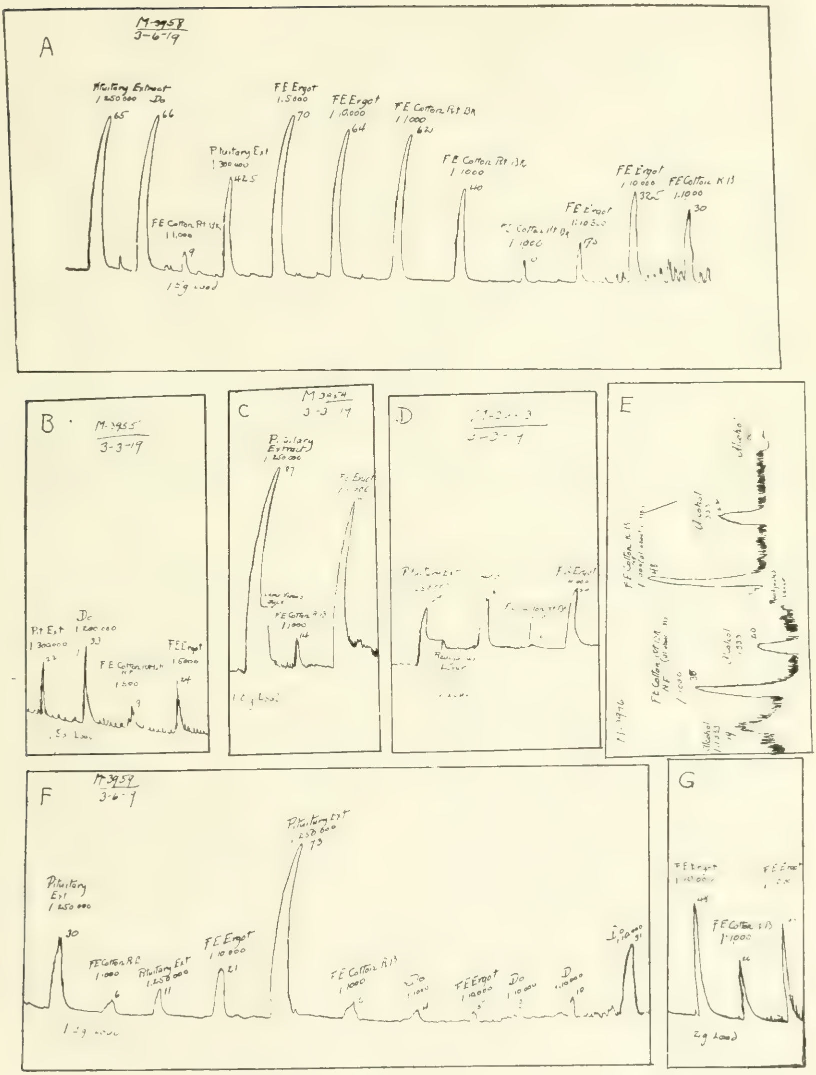
## CHART 2.

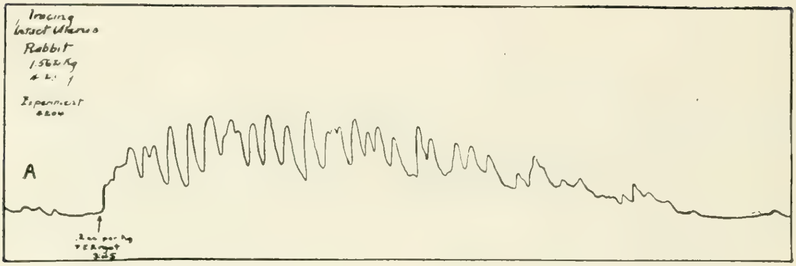
This chart shows the whole or a part of the kymograms taken in 7 experiments on the isolated, guinea pig's uterus. Contractions 7, 8, and 9 in A show the diminishing effect of succeeding doses of cotton root bark. Compare contraction 6 and 10 for the effect of ergot before and after cotton root bark. Note the first four contractions for comparison of cotton root bark with pituitary extract, and also kymograms B, C, D, and F. G and others show a comparison with ergot. F shows a diminished effect of pituitary extract after cotton root bark and an increased effect after ergot. E gives the effect of alcohol and shows that the effect of cotton root bark must be discounted to the extent of the effect of the alcohol.

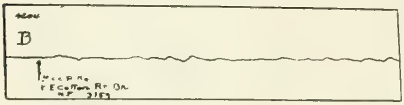
## CHART 3.

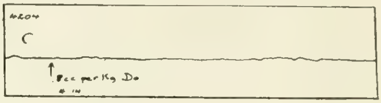
This chart shows sections of the kymograms of two experiments on the intact uterus of the virgin rabbit. In experiment 4202, D seems to show a diminished effect of ergot as compared with A, due to the two injections of cotton root bark shown in B and C. This experiment gives an idea of the relative effect of the two drugs. In experiment 4205, B shows the effect of cotton root bark to be diminished following A, even though the dose was increased.

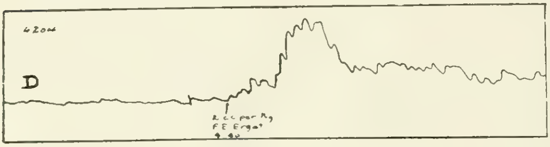

Kymogram 1294
11-5-15
150 mm.
236
215
140
Carotid Blood Pressure
Dog. Weight 14.1 Kg
5' after injection
10' after injection
½ minutes
Kymogram 778
128 mm
137
130
126
Carotid Blood Pressure
Dog. Weight 11.6 Kg
5' after injection
10' after injection
½ minutes
Kymogram 773
117 mm.
133
117
114
Carotid Blood Pressure
Dog Weight 8.3 Kg
5' after injection
10' after injection
½ minutes
Kymogram 779
138 mm.
152
135
140
Carotid Blood Pressure
Dog Weight 19.3 Kg
5' after injection
10' after injection
½ minutes

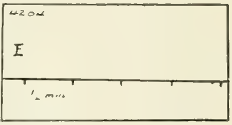

A
M-3958
3-6-19
Pituitary Extract 1/250000 65
Do 66
FE Cotton Rt Bk 1:1,000 9
15 g Load
Pituitary Ext 1/300000 42.5
FE Ergot 1:5000 70
FE Ergot 1/10,000 64
FE Cotton Rt Bk 1/1000 62
FE Cotton Rt Bk 1/1000 40
FE Cotton Rt Bk 1/1000
FE Ergot 1:10500 75
FE Ergot 1/10000 32.5
FE Cotton R B 1:1000 30
B
M-3955
3-3-19
Pit Ext 1/300000 22
Do 1/200000 33
FE Cotton Rt Bk NF 1/500 9
FE Ergot 1/5000 24
C
M 3954
3-3-19
Pituitary Extract 1/250000 87
FE Ergot
FE Cotton R B 1/1000 14
D
E
Alcohol
FE Cotton R B
Alcohol 333
FE Cotton R B NF 1/1000 38
Alcohol 1/333
F
M 3959
3-6-19
Pituitary Ext 1/250000 30
FE Cotton R B 1/1000 6
Pituitary Ext 1/250000 11
FE Ergot 1/10000 21
Pituitary Ext 1/250000 73
FE Cotton R B 1/1000
Do 1/1000
FE Ergot 1/10000
Do 1/10000
Do 1/10000 10
Do 1/10000 31
G
FE Ergot 1/10000 48
FE Ergot
FE Cotton R B 1/1000
2 g Load

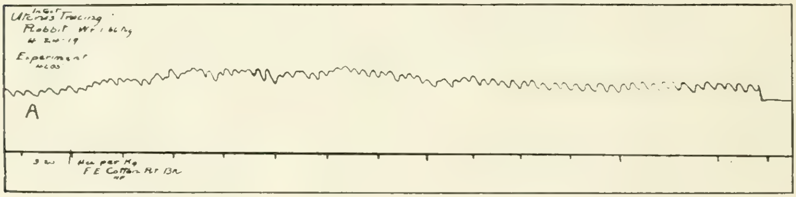

Tracing
Intact Uterus
Rabbit
1.562 Kg
4 2 1
Experiment
4204
A
.2 cc per Kg
F E Ergot
3:25

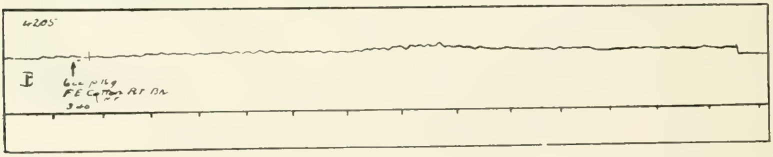

4204
B
cc p Kg
F E Cotton Rt Br
H F 3:59

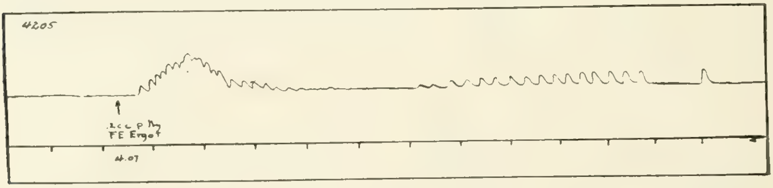

4204
C
cc per Kg Do
4 14

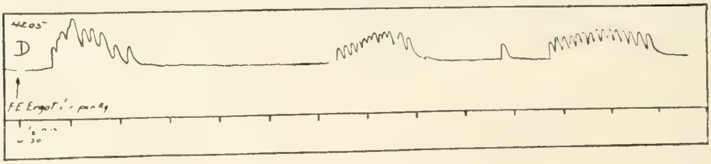

4204
D
.2 cc per Kg
F E Ergot
4 40


4204
E
½ min


Intact Uterus Tracing
Rabbit Wt 1.66 Kg
4 20 19
Experiment
4205
A
3 20
cc per Kg
F E Cotton Rt Br
H F


4205
B
6 cc p Kg
F E Cotton Rt Br
H F
3 40


4205
.2 cc p Kg
FE Ergot
4.07


4205
D
FE Ergot per Kg
½ min
4 30

# THE SPECIFICITY OF DISINFECTANTS AND ITS BEARING ON THEIR STANDARDIZATION

A. L. WALTERS, B. S., M. D.

[Read before the Laboratory Section, American Public Health Association, October 20, 1917, at Washington, D. C., and reprinted from the American Journal of Public Health, Vol. VII, No. 12.]

The standardization of disinfectants has been an annual subject of discussion before health congresses and societies since the advocation of the "drop method" proposed in 1903 by Rideal and Walker. During these years many refinements, changes and additions have been made to this method, the latest being the control of the acidity of the media by determining the H-ion concentration. There is no doubt that the method has been improved but the question arises, has not the broad purpose of the test been overlooked in searching out the details?

There was a time when with limited bacteriological knowledge, it was reasonable to suppose that all disinfectants would bear the same germicidal relation to phenol, no matter on what organism tested. This time has now passed and when a label bears the statement that a certain preparation has a phenol coefficient of 2, it cannot be taken literally, but must be interpreted in the light of the method used in determining this coefficient. If this method is that of the hygienic laboratory it means that this preparation under the conditions of that test is twice as germicidal as phenol *on the bacillus typhosus, or a particular strain thereof.* No conclusions should be drawn as to its germicidal action on the staphylococcus, streptococcus or any other organism, not excepting the bacillus coli, or paratyphosus. Naturally the closer the chemical relation between the disinfectant and phenol, and the closer the bacteriological relation of the organisms concerned in the comparison, the more nearly correct will be any conclusion drawn from such a test. On the other hand, it is only rational to suppose that disinfecting agents of widely different character will vary greatly in their germicidal relation to phenol when tested on organisms of different genera or species. Since the advent of synthetic dyes in preparing differential culture media and later the production of diarseno-benzol, the specificity of drugs for certain microorganisms should not be lost sight of. Chick and Martin (1) recognize this specificity of disinfectants when they say, "Some disinfectants are more efficient against a particular organism than against any other." Churchman's (2) work was quite definite in showing the selective affinity of gentian violet for certain organisms. In the main he could divide bacteria into two classes, those which were inhibited in their growth by very weak dilutions of this dye and those which were unaffected by even much stronger solutions. As a rule closely related bacteria reacted similarly to gentian violet but he found a marked exception to this in one strain of B. enteritidis. This strain was indistinguishable by ordinary cultural and straining methods from four other strains of B. enteritidis, yet it was completely inhibited by weak dilutions (1:80,000) of gentian violet, whereas the other four were not. Churchman states that this instance is significant because it establishes the fact that chemical substances may be so specific in their selective affinity for microorganisms as to distinguish among strains of bacteria otherwise indistinguishable; and further that it indicates to what extent our ideas of bactericides must be modified by the conception of chemical affinity, and that the fact that a given bactericide kills a given organism does not justify the conclusion that it will kill all closely related organisms, or even all strains of the same organism.

Browning and Gilmour (3) examined a number of organic and inorganic compounds for their bactericidal action toward different species of bacteria, with a view to discovering (a) substances possessing specific bactericidal properties for particular organisms and—(b) relationships between chemical constitution and bactericidal action. They found staphylococcus aureus and B. anthracis, organisms more resistant to phenol than is B. typhosus, less resistant than the coli-typhoid group of organisms to the action of certain basic benzol derivatives among which may be mentioned fuchsin, hexamethyl violet, methylgreen, malachite-green and brilliant green. The amount of hexamethyl violet required to inhibit B. typhosus was 150 times that required for staphylococcus aureus. Furthermore these authors state that whereas the action of malachite green shows no marked difference between its effect on B. typhosus and B. coli, brilliant-green exerts a more marked bactericidal action on B. coli than on B. typhosus. In other words, the bactericidal action of a disinfectant on one organism is not necessarily a measure of its action on another, even when these organisms belong to the same genus or closely related group. Another very interesting feature of Browning and Gilmour's work is that they found the presence of serum to increase the antiseptic action of flavine for both staphylococcus and B. typhosus. This is certainly contrary to the action of serum on the effect of any other class of disinfectants and should be taken into consideration when giving them a valuation of phenol coefficient.

Flavine is an example of a valuable disinfectant which if gauged by its phenol-coefficient would be practically worthless. Pine oil, on the contrary, is an example of one which, based on its phenol coefficient, should be of considerable importance, but which in practice is deficient in some respects as a general disinfectant. This deficiency was brought to my attention after the issuance of the hygienic laboratory's report (4) on pine oil as an efficient liquid disinfectant when I was asked by a dentist how long a time would be required to sterilize instruments in this Pine Oil Disinfectant. The Pine Oil Disinfectant was made by incorporating 62.5 per cent. of pine oil in soap according to the directions for making the "Hygienic Laboratory Pine Oil Disinfectant." The phenol coefficient of this solution was determined and found to be 3.8. Several different samples of pine oil purchased on the open market varied in their phenol coefficient. This variability of pine oil was mentioned by Hamilton (5) before this association last year.

In testing the ability of this Pine Oil Disinfectant to sterilize dental or other instruments, small steel blades (scarifiers). ½x3½ centimeters were contaminated with various organisms and subjected to the action of the disinfectant, using phenol as a control, in the following manner:

### Experiment I.

Twenty-four hour broth cultures of recently isolated and old stock cultures of staphylococcus aureus were each used to inoculate 84 metal scarifiers. Both lots were placed in sterile petridishes to dry over night.

## TABLE I.

### CULTURES OF STAPHYLOCOCCUS AUREUS—DRIED.

Recently Isolated Cultures.

| Time | 2% pine oil disinfectant | 4% pine oil disinfectant | 5% phenol | Water control |
|---|---|---|---|---|
| 5 minutes | + + + | + + — | + — — | + + + |
| 10 minutes | + + + | + + + | — — — | + + + |
| 15 minutes | + + + | + + + | — — — | + + + |
| 30 minutes | + + — | + + + | — — — | + + — |
| 1 hour | + + — | + + — | — — — | + + + |
| 3 hours | — — — | — — — | — — — | + + + |
| 20 hours | — — — | — — — | — — — | + + + |

Old Stock Cultures.

| Time | 2% pine oil disinfectant | 4% pine oil disinfectant | 5% phenol | Water control |
|---|---|---|---|---|
| 5 minutes | + + + | + + + | — — + | + + + |
| 10 minutes | + + + | + + + | + + — | + + + |
| 15 minutes | + + + | + + + | — — + | + + + |
| 30 minutes | + + + | + + + | — — + | + + + |
| 1 hour | — — + | + + — | — — — | + + + |
| 3 hours | — — — | + + — | — — — | + + + |
| 20 hours | — — — | — — — | — — — | + + + |

## TABLE II.

### CULTURES OF STAPHYLOCOCCUS AUREUS—NOT DRIED.

Recently Isolated Cultures.

| Time | 2% pine oil disinfectant | 4% pine oil disinfectant | 5% phenol | Water control |
|---|---|---|---|---|
| 5 minutes | + + + | + + + | — — — | + + + |
| 10 minutes | + + + | + + + | — — — | + + + |
| 15 minutes | + + + | + + — | — — — | + + + |
| 30 minutes | + + + | + — — | — — — | + + + |
| 1 hour | + + — | + + — | — — — | + + — |
| 3 hours | + — — | — — — | — — — | + + + |
| 18 hours | — — — | — — — | — — — | + + + |

Old Stock Cultures.

| Time | 2% pine oil disinfectant | 4% pine oil disinfectant | 5% phenol | Water control |
|---|---|---|---|---|
| 5 minutes | + + + | + + + | + — — | + + + |
| 10 minutes | + + + | + + — | — — — | + + + |
| 15 minutes | + + + | + + — | + — — | + + + |
| 30 minutes | + + + | + + + | — — — | + + + |
| 1 hour | + — — | — — — | — — — | + + + |
| 3 hours | — — — | — — — | — — — | + + + |
| 18 hours | — — — | — — — | — — — | + + + |

With sterile distilled water, 2 per cent. and 4 per cent. emulsions of Pine Oil Disinfectant and 5 per cent. solution of phenol were made. Sterile distilled water was used as control. Five per cent. phenol was taken because it is the strength commonly used in sterilizing instruments.

The scarifiers were placed in the above solutions and also in sterile water as a control, 21 in each, and allowed to remain for periods of 5-10-15-30 minutes, 1 hour, 3 hours, and over night. Those placed in the Pine Oil Disinfectant were removed, 3 at the end of each priod of time, washed in sterile water and placed in fermentation tubes of broth.

Those in 5 per cent. phenol were removed and washed in 95 per cent. alcohol before placing in fermentation tubes. Those in sterile water were placed directly in fermentation tubes without washing.

After 24 hours' incubation, transplants were made from the fermentation tubes to agar slants. The results are given in Table I in which the plus sign indicates growth of the organisms. The three columns following each interval of time represent the three scarifiers used in each solution.

By the hygienic laboratory method of testing disinfectants, Pine Oil Disinfectant would be classed as having 4 times the germicidal power of phenol. From the above experiment in which staphylococci were used as test organisms, Pine Oil Disinfectant, 2 per cent. or even 4 per cent. does not destroy the organisms in one hour; whereas they are usually killed in 5 per cent. phenol within 15 minutes. Another striking point brought out in Table I is that Pine Oil Disinfectant, 4 per cent., is no more effective than the 2 per cent. dilution.

In order to check these results a second series was carried out differing from the former only in that the inoculated scarifiers were not dried before placing them in the disinfectant solutions and that the fermentation tubes were incubated 48 hours before transplants were made from them.

These results confirm those of Experiment I, and again show that Pine Oil Disinfectant diluted with water to make a 4 per cent. mixture or emulsion is little, if any, more effective than the 2 per cent. emulsion.

Other similar tests were made using B. typhosus, streptococcus, pyogenes, and B. diphtheria and showed that in practically all instances the test organisms were killed within 15 minutes by the disinfectants employed; and that of the four bacteria used in the experiments, the staphylococcus alone stood out as being disproportionately resistant to pine oil solutions in soap.

Pine oil cannot, then, be termed a general disinfectant of high germicidal value, for although showing to advantage when tested on the typhoid bacillus, it is much less efficient than phenol or cresol when the infecting organism is the staphylococcus aureus. This is another instance of the fallacy of the present generally accepted method of determining the value of disinfectants, namely, the so-called Hygienic Laboratory Method. The physician or dentist who does not know the intricacies or inaccuracies of the present methods of testing or standardizing disinfectants is justified in thinking that a phenol coefficient of 4 means that the preparation is four times as active as phenol, and that 1 or 2 per cent. solution of it will sterilize his instruments within 5 or 10 minutes. He is further justified in reposing confidence in the phenol coefficient of a disinfectant because the method of determining this is sanctioned by the government and by the Council on Pharmacy and Chemistry of the American Medical Association. One state requires that disinfectants must have the phenol coefficient on the label, and before a disinfectant will be admitted to New and Non-Official Remedies, this same condition must be complied with. Defects of this method have been repeatedly pointed out, but have been apparently disregarded by its advocates and entirely overlooked by others interested in having it made a legal method for standardizing disinfectants. Hamilton and Ohno (6) have shown that this method in different hands gives widely varying results when testing the same disinfectant on the same strain of typhoid organism. If comparable results cannot be obtained when the same organism is used, one would certainly expect a disinfectant to vary widely in germicidal power when tested on different organisms. In fact, Anderson and McClintic, the originators of the Hygienic Laboratory Method, say (7) "Unless different observers use the same species of organism, there can be no possibility of uniformity in results. The coefficient obtained with different species may vary as much as 300 per cent." If this be the case, what is the practical value of the phenol coefficient as determined by the Hygienic Laboratory Method? Certainly only a small proportion of any commercial disinfectant is employed to destroy typhoid bacilli.

In the case of pine oil, we have an agent considerably more germicidal than phenol when acting on the typhoid bacillus, and very much less active than phenol on the staphylococcus. This proves conclusively that the comparative disinfectant value of pine oil and phenol cannot be determined by any one test.

Other defects in the Hygienic Laboratory Method have been pointed out. Duyser and Lewis (8) call attention to three factors which cause unreliable results. As stated they are "(a) The use of an excessive number of bacteria depletes the disinfecting solution before the culture is rendered sterile. (b) An unknown volume is withdrawn for testing, in that the volume of the standard loop is not constant. (c) It is impossible to determine from the broth tube inoculated, how complete the killing was at the time the sample was withdrawn." Duyser and Lewis showed that the so-called standard loop, which is supposed to transfer a constant amount of culture from a tube or flask to the disinfectant, varies under the best conditions at least 30 per cent. from the average, and when not very carefully used may vary as much as 80 per cent. This inaccuracy of the standard loop was verified by A. D. St. John (9) who was able to obtain with the same loop a variation of 300 per cent. by slowly withdrawing the loop edgewise and quickly withdrawing it parallel with the surface. The lack of uniformity of different loops in the hands of different bacteriologists can readily be appreciated.

So that, as a matter of fact, the stating of the phenol coefficient on a label may be misleading and, instead of informing the public as to the value of any particular agent, may misinform and give a false sense of security. At the same time it would afford ample opportunity for litigation and a wide difference of opinion among experts.

The value of any method of testing disinfectants has been well stated by Phelps (10), "The results of any disinfection experiments are fundamentally influenced by such conditions as temperature, character of the organism employed, number of organisms in unit volume, and character of the medium. In the absence of complete data covering these points results are practically worthless, at least for purposes of comparison. Even with such data given, it is still impossible owing to the variable conditions obtaining in practice, to establish any relationships, or order of excellence, among the various disinfectants. At best we can only hope to establish such relationship under specified experimental conditions." He further says "It seems at present to be quite necessary to determine the relative germicidal values of two disinfectants upon the actual kind of germ upon which they are to be employed and to qualify the final comparative results accordingly." In no case has the practical value of this dictum been so clearly exemplified as in the present investigation. Based on the report of Stevenson from the Hygienic Laboratory entitled "An Efficient Liquid Disinfectant," one would be led to think that "Pine Oil Disinfectant" is 4 to 6 times as effective a germicide as phenol, without any exceptions. That this is not the case has been clearly shown by the preceding experiments and contrary to the statement in the above report, Pine Oil Disinfectant cannot be satisfactorily used to replace the ordinary coal tar compounds commonly employed as disinfectants.

There is another phase of the question brought up by these discrepancies, namely, legalizing the Hygienic Laboratory Method as a standard method under which prosecutions may take place. That this is not desirable is readily understood when the inaccuracy of the method is considered. Almost every factor concerned is variable and is difficult or impossible to control. The temperature, the media, the number of bacteria used, the change in resistance of the organisms, and the technique are some of these factors already mentioned. These have been well discussed in the paper of J. T. Ainslee Walker (11).

Aside from these considerations, the true value of a disinfectant can only be stated in terms of the organism or organisms on which it is to be used in actual practice. To state that Pine Oil Disinfectant is "an efficient liquid disinfectant" which "may be used wherever the ordinary coal tar compounds are used" is certainly drawing erroneous conclusions from a phenol coefficient determination.

The paradoxical action of pine oil in being markedly more germicidal than phenol on the typhoid bacillus and decidedly less germicidal on the staphylococcus is an interesting phenomenon deserving further study and is apparently illustrative of specificity in disinfectants.

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(1) Chick and Martin. Jr. Hygiene, 1908, 8, 654.
(2) Churchman, Jr. Exper. Med., 1912, 16, 221 and 822.
(3) Browning and Gilmour, Jr. Path. and Bact., 1913, 18, 144.
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(5) Hamilton. Am. Jr. Public Health, 1917, 7, 282.
(6) Ohno and Hamilton. Am. Jr. Public Health, 1914, 4, 486.
(7) Anderson and McClintic. Jr. Infectious Diseases, 1911, 8, 1.
(8) Duyser and Lewis. Jr. Industrial and Engineering Chem., 1914, 6, 198.
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(11) Walker. N. Y. Medical Jr., 1916, 103, 500.

# THE PREPARATION OF 1,2-DICHLORO-ETHER.

BY E. A. WILDMAN AND HAROLD GRAY.

[Reprinted from the Journal of the American Chemical Society, Vol. XLI. July, 1919.]

An investigation which was recently carried on involved the use of 1,2-dichloro-ether, a substance which is not available on the market. An examination of the literature showed that the best method of preparation was probably, by direct chlorination of ether, a method used by Fritsche and Schumacker,[1] although they did not give sufficient details to enable one to carry out the operation successfully.

The ease with which chlorine and ether react is shown by the fact that a mixture of the two in gaseous form may ignite spontaneously. If a flask is filled with chlorine at room temperature and one or two cc. of ether poured in, there will usually follow within a moment a slight explosion with ignition of the ether and the deposition of a quantity of soot. If during the chlorination of ether, particularly at the first, the temperature is not kept low by cooling with an ice-water bath, and the chlorine run in slowly there is likely to be an accumulation of chlorine above the liquid which will result in the sudden ignition of the whole mass. As the reaction progresses the ether becomes saturated with hydrogen chloride which decreases its inflammability and cooling is no longer necessary. The rate of passing in chlorine may also be much increased for the reaction takes place more rapidly than at first.

There is another difficulty which enters when the ether becomes saturated with hydrogen chloride. The latter seems to dissolve to the extent that it forms a supersaturated solution when it suddenly escapes with almost explosive violence, carrying the ether along with it. In one case when 6 liters of ether were being chlorinated in a large wide-mouthed bottle the entire contents with the exception of about 500 cc. was ejected as if from a geyser. Occasional agitation of the material was not sufficient to prevent this. It was found necessary to agitate continuously by means of a rapidly revolving mechanical stirring device.

The operation was carried out as follows:

A two-liter flask was provided with a return condenser, an inlet tube for chlorine and an efficient stirring device connected by means of an oil seal in order that the escaping hydrogen chloride would be forced to go through the condenser. 800 g. of dry ether was placed in the flask and kept cold by a surrounding bath of ice and water. The ether was stirred from the beginning. After passing in a slow stream of chlorine for 35 hours the specific gravity was 0.785 at 25° and hydrogen chloride began to be evolved freely. The temperature was then allowed to rise gradually to that of the room. At the end of 65 hours the specific gravity was 0.89, and after 82 hours, 0.96. Chlorination was stopped at this point.

The unchanged ether was distilled off under reduced pressure and the remaining material fractionated. There was obtained 375 g. of dichloro-ether, boiling from 66 to 69° at 45 mm., representing a yield of 24.2% of the theoretical amount. The material in the distilling flask remained colorless till near the end, when it became black and there was left a small residue of 65 g.

It had previously been determined that chlorination should be stopped at about the point where the specific gravity was 0.96. In one trial during which it was carried on to the specific gravity 1.11 it was impossible to separate a pure fraction of dichloro-ether from the product.

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SERIES 2 NUMBER 1

# THE LILLY SCIENTIFIC BULLETIN

INTESTINAL EXTRACTION OF ADSORBED ALKALOIDS

NEW BENZYL ESTERS POSSESSING AN ANTISPASMODIC ACTION

ATROPINE SULFATE FROM DATURA STRAMONIUM

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# THE LILLY SCIENTIFIC BULLETIN

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# INTESTINAL EXTRACTION OF ADSORBED ALKALOIDS

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and
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The purpose of this work is to determine whether alkaloids adsorbed on Lloyd's reagent, a variety of fullers' earth, are extracted from their adsorption compounds in the intestinal tract and thus rendered therapeutically available. It may be stated as axiomatic that animal experiments still afford the only reliable means of determining whether an adsorbed drug, administered orally, is or is not assimilated in the intestinal tract.

Nevertheless, in a recent publication on intestinal ipecac preparations, Sollmann[1] inferred from test tube experiments, unsupported by experiments on animals, that the ipecac alkaloids when administered orally in the form of "Alcresta Ipecac" (an adsorption compound of the ipecac alkaloids on Lloyd's reagent) are not released and absorbed in the intestinal tract and are consequently therapeutically "inactive." Sollmann did not employ intestinal contents or the extract of intestinal mucosa as the extracting medium, but confined his experiments to the use of distilled water or water rendered very slightly alkaline by the addition of 1 per cent. sodium bicarbonate, with and without the addition of bile salts, and found that these solutions failed to extract any appreciable amount of alkaloid from the adsorption compound. He stated that "It appeared from these experiments that the assumption of the intestinal liberation of emetin from alcresta ipecac is untenable."

Sollmann did not refer to the published results of Bass,[2] Stephens[3] and others on the favorable action of adsorbed ipecac in pyorrhea, dysentery, etc., and concluded his paper with

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[1] Sollmann, Torald; The Solubility of Intestinal Ipeac Preparations, J. A. M. A. 71: 1125 (Oct. 11) 1919.

[2] Bass, C. C., and Johns, F. M.: Pyorrhea Dentalis and Alveolaris, J. A. M. A. 64: 553 (Feb. 13) 1915.

[3] Stephens, J. W. W., and Mackinnon, Doris L.: A Preliminary Statement on the Treatment of Entamoeba Histolytica Infections by "Alcresta Ipecac," Ann. Trop. Med. & Parasitol. 10: 397 (Feb.) 1917; Protozoological Investigation of Cases of Dysentery Conducted at the Liverpool School of Tropical Medicine, ibid: 11: 27 (June) 1917.

the statement that "the pharmacologic literature of alcresta ipecac confirms that the emetin remains entirely inactive."

To understand the significance attached by Sollmann to the factor of intestinal alkalinity in this connection, it is necessary to review briefly the history of this subject.

The use of the adsorption compound of the ipecac alkaloids for therapeutic purposes followed the observations of J. U. Lloyd[4] that alkaloids were actively adsorbed from acid aqueous solutions by means of fullers' earth, and liberated by ammoniacal chloroform. Since stomach contents are acid, it appeared probable that the emetic effect following oral administration of the ipecac alkaloids might be avoided by administering the adsorption compound of the alkaloids on Lloyd's reagent, the adsorbed alkaloid passing through the acid stomach contents unchanged, but being extracted on reaching the intestinal tract. This proved to be the case, many times the emetic dose of the alkaloids have been taken in this form without producing either vomiting or nausea.

Therapeutic effects following the use of this preparation were noted by various investigators in dysentery and subsequently in pyorrhea, etc., and these effects, as well as the diarrhea induced by too large a dose of the adsorbed ipecac alkaloids, afforded at least presumptive evidence of their release in the intestinal tract.

In attempting to explain the release of the ipecac alkaloids from their adsorption compound in the intestinal tract, Eli Lilly and Company and other investigators appear to have attached too much importance to the factor of intestinal alkalinity, and to have overlooked the possible rôle of organo-extractives and protoplasmic constituents of the cells of the intestinal mucosa in effecting the release in question. Sollmann's recent demonstration that the theory of extraction of alkaloids by weak aqueous alkalis is untenable does not, however, constitute a refutation of the clinical and pharmacologic data on which the theory was based.

In the subsequent experimental section it will be demonstrated that:

1. Sollmann's experiments do not justify the deductions drawn therefrom.
2. Not only emetin, but also quinin, strychnin and atropin, administered by mouth in the form of their adsorption compounds with Lloyd's reagent, from which they are not released by extraction with water or weak alkali, are absorbed in the intestinal tract and produce pharmacologic effects that can only be attributed to the specific alkaloids themselves.
3. The alkaloids in question are released from their adsorption compounds when the latter are introduced into the

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[4] Lloyd, J. U.: Discovery of the Alkaloidal Affinities of Hydrous Aluminum Silicate, J. Am. Pharm. Assn. 5: 381, 1915.

neutral peritoneal cavity, from which it must be concluded that protoplasmic extraction of the alkaloids proceeds in the absence of alkalinity.

4. Furthermore, soaps and other substances occurring in the intestinal tract and in living protoplasm extract the alkaloids from their adsorption compounds with comparative facility in either a neutral or a faintly alkaline solution.

## EXPERIMENTAL

For the sake of convenience, the experimental section of this paper will be divided into two portions:

1. A pharmacologic demonstration that emetin, quinin, strychnin and atropin, when present in an adsorbed state in Lloyd's reagent, are released in the intestinal tract or the peritoneal cavity, producing pharmacologic effects which can only be attributed to the alkaloids themselves.

2. A physical and chemical demonstration that the alkaloids in question may be extracted from their adsorption compounds by means of soaps, lipoids and other substances occurring in the intestinal contents or normally present in protoplasm, even though the alkalinity does not exceed that set by Sollmann and others as the level for intestinal contents, thus affording a satisfactory physicochemical explanation as to how the alkaloids are absorbed by the body cells, although not extracted by water.

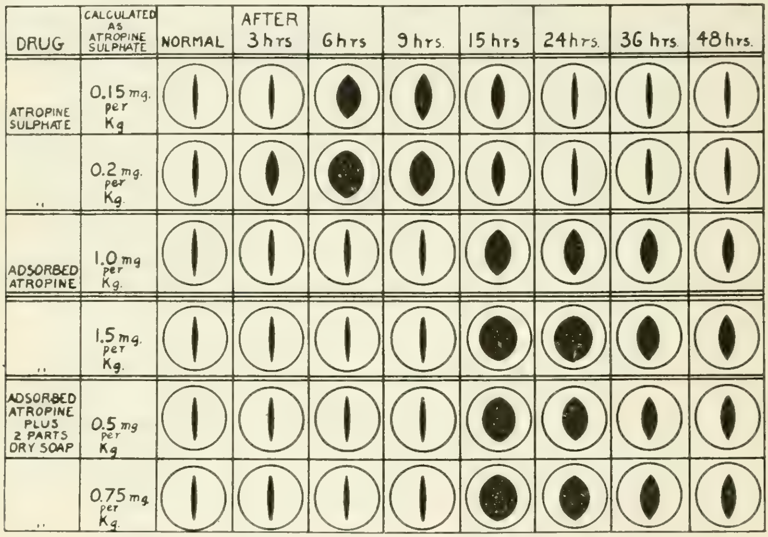
It should be noted that throughout these experiments, unless otherwise stated, adsorption preparations of the alkaloids on Lloyd's reagent were employed containing 4 per cent. or less of the alkaloid. These preparations had been exhaustively washed with water prior to commencement of the experiments, until no trace of alkaloid was present in the wash water. On testing they were found to yield no alkaloid in acid aqueous solutions having approximately the same concentration of acid as the stomach contents, and only such negligible traces as Sollmann himself observed when they were extracted with such weak alkaline solutions as the 1 per cent. sodium bicarbonate which Sollmann selected as his standard for intestinal contents.

Since it is difficult to measure the effects produced by means of emetin, it was decided to carry out the initial experiments on animals with atropin and strychnin on account of the facility with which the characteristic effects of these alkaloids may be recognized.

### ATROPIN

Adsorbed atropin given by mouth to cats causes full dilatation of the pupils with failure to react to light. Control experiments on the same animals with atropin sulphate indicate that approximately seven times as much adsorbed atropin as free atropin is required in order to produce mydriasis. A very important observation is that the adsorbed atropin exerts its effect less rapidly; the effect, however, being more uniform and lasting much longer, a point of considerable clinical importance. Furthermore, as an indicator of the mechanism whereby the atropin is extracted from its adsorption compound, it is interesting to note that the amount of adsorbed atropin required to produce mydriasis is lowered 50 per cent. if a small amount of neutral soap is given with the adsorbed atropin when administered orally.

While cats exhibit variations in their sensitivity to atropin sulphate and adsorbed atropin administered orally, the relative proportions of these substances required to produce comparable results in individual cats are approximately the same.



Mydriatic effect of atropin given orally to a cat

The accompanying diagram, based on results obtained with the same animal, illustrates the extent to which dilatation of the pupil is maintained when subjected to a bright light, thus affording an index of atropin effect at various periods of time after the administration of the alkaloid. From these experiments it is immediately apparent that the adsorption compound, which fails to yield any alkaloid when tested on the basis proposed by Sollmann, nevertheless gives up a sufficient proportion of alkaloid to the intestinal mucosa to produce full dilatation of the pupil with complete failure to react to strong light. It is extremely interesting to note the delay in development of the maximum effect and the prolongation of effect as compared with results obtained with the free alkaloid.

Adsorbed atropin introduced directly into the conjunctival sac produced a typical dilatation of the pupil of the treated eye.

## STRYCHNIN

The adsorbed strychnin preparation employed in these preliminary experiments contained 4 per cent. strychnin and yielded no alkaloid on extraction with water or 1 per cent. sodium bicarbonate. This preparation induced typical strychnin effects when administered orally to rabbits, the minimum lethal dose representing 20 mg. strychnin sulphhate per kilogram of rabbit. It was found that the adsorbed strychnin in this preparation possesses about one eleventh of the toxicity of free strychnin, but it is interesting to note that, as in the case of atropin, when dry soap is given at the same time the toxicity is doubled, from which it must be concluded that the soap facilitates the release of the strychnin from its adsorption compound and its passage into the mucosa.

The lethal dose is considerably diminished by using adsorption compounds containing more than 4 per cent. of strychnin, although none of these compounds yield alkaloid on extraction with water or 1 per cent. sodium bicarbonate. Seven and seven-tenths mg. of strychnin sulphate administered in the form of the 7.5 per cent. adsorption compound exerted an effect equal to 20 mg. in a 4 per cent. compound, and to 1.8 mg. free strychnin sulphate.

TABLE 1.—MINIMAL LETHAL DOSE FOR RABBITS EXPRESSED AS STRYCHNIN SULPHATE

| | Mg. per Kilogram |
|---|---|
| Free strychnin sulphate | 1.8 |
| 7.5 per cent. adsorbed strychnin | 7.7 |
| 4 per cent. adsorbed strychnin | 20.0 |
| 4 per cent. adsorbed strychnin with two parts dry soap | 10.0 |

The animals which received lethal doses of adsorbed strychnin exhibited characteristic strychnin symptoms, hypersensitiveness followed by convulsions and death within four or five hours. While the foregoing experiments demonstrate that extraction by means of a 1 per cent. sodium bicarbonate solution affords no index regarding intestinal absorption, they do not settle the question as to whether intestinal alkalinity or even the intestinal contents themselves are factors of primary importance in effecting the release of the alkaloids in the intestinal tract.

Previous experiments carried out with adsorbed strychnin and marine larvae at Woods Hole in the summer of 1919, suggested the possibility that the alkaloid may be extracted directly from its adsorption compound by protoplasmic constituents of the cells of the intestinal mucosa.

To insure approximate neutrality of the tissues and solutions involved, and to rule out the intestinal tract and its contents entirely, experiments were carried out in which the above mentioned preparations of adsorbed strychnin were introduced directly into the peritoneal cavities of rats. The lethal doses were found to be approximately 11 mg. of strychnin sulphate for the 4 per cent. adsorption compound, and 6.4 mg. of strychnin sulphate for the 7.5 per cent. compound, per hundred grams of rat; the animals receiving these doses exhibited typical strychnin symptoms and died in two or three hours. The dose of strychnin sulphate required to cause death when introduced into the peritoneal cavity in a single dose was found to be from 0.2 to 0.3 mg. per hundred grams, or approximately one-fortieth the amount present in the lethal dose of the 4 per cent. adsorbed strychnin. This does not, however, afford a fair basis of comparison, since the free strychnin sulphate is rapidly absorbed and exhibits a high peak effect, while there is every reason to believe from the experiments on atropin, presented above, that the alkaloid is given off from the adsorption compound slowly, evenly and with far less peak effect.

TABLE 2.—STRYCHNIN INTRAPERITONEALLY IN RATS

| Drug | Dose per 100 Gm. Rat Calculated as Strychnin Sulphate, Mg. | Results |
|---|---|---|
| Strychnin sulphate, single dose | 0.175 | Survived |
| | 0.2 | Death in 75 minutes |
| Strychnin sulphate, divided doses: | | |
| 0.065 mg. every 15 min. | 0.455 | Died after 7 injections in 1¾ hours |
| 0.065 mg. every 20 min. | 0.455 | Died after 7 injections in 2 hours. |
| 0.065 mg. every 30 min. | 0.975 | Survived after 15 injections in 7 hours |
| 4 per cent. adsorbed strychnin | 10 | Convulsions in 1 hour; recovered |
| | 11 | Convulsions and death in 2 hours |
| 7.5 per cent. adsorbed strychnin | 5 | Survived |
| | 6.4 | Convulsions and death in 1 hour |
| 4 per cent. adsorbed strychnin in collodion sack | 12 | No effect in 24 hours: sack opened; death in 2 hours |
| | 50 | No effect in 4 days; sack opened; death in 1 hour |
| Strychnin sulphate in collodion sack | 0.5 | Death in 5 minutes |

A fairer basis of comparison is afforded by the use of divided doses of strychnin sulphate, fractional amounts being administered at from fifteen to thirty minute intervals; the total dose required to cause death in from two to three hours being determined. Working on this basis, rats weighing 100 gm. were found to withstand the intraperitoneal adminstration of 0.13 mg. of strychnin sulphate per hour without convulsions, but doses of from 0.2 to 0.25 mg. per hour proved fatal at the end of from one and three-fourths to two hours. From this it may be concluded that 0.5 mg. of free strychnin sulphate, when administered in divided doses, is about equivalent to 11 mg. of strychnin sulphate in the form of the 4 per cent. adsorbed strychnin, and 6.4 mg. of 7.5 per cent. material, giving ratios of 20:1 and 13:1, respectively, figures which correspond roughly with extraction data to be presented subsequently.

It may be concluded from the foregoing that alkalinity is not necessary for cellular extraction of strychnin from its adsorption compound.

In order to determine to what extent direct contact between the protoplasmic surfaces of the peritoneum and the adsorption compound was a necessary factor for the extraction or release of the alkaloid, collodion sacks were prepared containing a sufficient proportion of glycerin to render them freely permeable to strychnin or soap, and moderately permeable to albumin, but still impervious to cells or particles of Lloyd's reagent (Table 2). Lethal doses of the 4 per cent. adsorbed strychnin preparation were then introduced into the sacks, which were sealed and placed in the peritoneal cavities of rats. No toxic effects whatever were observed for a period of two or three days. The sacks were then exposed, cut open and the peritoneal cavity once more closed. In all such cases even a minimal fatal dose, after remaining two or three days in the sack, during which time it had exerted no strychnin effect, was found when the sack was opened to possess its full toxicity, the death of the rat occurring just as rapidly as would have been the case had a corresponding amount of adsorbed strychnin been introduced into the peritoneal cavity, from which it must be concluded that during the period that the adsorbed strychnin remained in the sack, there had been no loss of strychnin through the sack wall. Since the sacks permitted a comparatively free diffusion of aqueous fluids, and control tests showed that strychnin sulphate passed through with sufficient rapidity to cause the death of animals in a few minutes, these experiments afford some support of the view that the release of strychnin from its combination with Lloyd's reagent, both in the peritoneal cavity and in the intestinal tract, is to be attributed primarily to direct contact between the adsorption compound and the protoplasm of the cell, and is not dependent on the alkalinity of the intestinal contents.

## QUININ

An adsorbed quinin preparation, containing 4 per cent. of the alkaloid and yielding no quinin on extraction with water, was administered orally to dogs, the amount excreted in the urine being determined with as much accuracy as possible. Quinin sulphate was given orally to the same dogs for control purposes.

The low recovery of quinin in the urine is probably attributable to oxidation. It is interesting to note that in the case of quinin, the alkaloid is extracted from its adsorption compound and is excreted in amounts comparable to those following the administration of quinin sulphate.

TABLE 3.—EXCRETION OF QUININ WHEN ADMINISTERED AS QUININ SULPHATE AND AS ADSORBED QUININ

| | Quinin Sulphate, Per Cent. | Adsorbed Quinin. Per cent. |
|---|---|---|
| Dog 1 | 21.4 | 19.8 |
| Dog 2 | 30.9 | 19.3 |

## EMETIN

Emetin as a free alkaloid or alkaloidal salt does not lend itself to experiments designed to compare its effect with those exerted by the Alcresta tablets of ipecac or emetin adsorbed on Lloyd's reagent because the free emetin cannot be given orally without producing emesis. When Alcresta tablets of ipecac which yield no alkaloid on extracting with water, are administered orally to dogs in fairly large doses, a diarrhea is produced which affords evidence that some of the alkaloid is released in the intestinal tract, since Lloyd's reagent alone is not a laxative.

Furthermore, following oral administration of as small a dose as 2 grains of adsorbed emetin the alkaloid may readily be detected in the urine. In all experiments with dogs, some emetin has been recoverable in the urine.

The ipecac aklaloids are much less toxic than strychnin and their effect is more delayed and is not accompanied by any readily recognized symptoms; consequently it is difficult to parallel the adsorbed strychnin results in the intestinal tract and peritoneal cavity. By using a 10 per cent. adsorption compound of emetin on Lloyd's reagent, a preparation which yields no emetin on extraction with water or 1 per cent. sodium bicarbonate, lethal effects were exerted on cats to which the preparation was administered orally, and on rats when it was introduced into the peritoneal cavity. Emetin appears to be absorbed very slowly from the intestinal tract, but exerts a cumulative effect, causing lethargy and loss of appetite, death occurring in four or five days after oral administration of the 10 per cent. adsorption compound to cats in a dose of 60 to 70 mg. of adsorbed emetin per kilogram of body weight. Similarly, 20 mg. of emetin in the form of the 10 per cent. adsorption compound, when introduced into the peritoneal cavity of rats weighing 100 gm., causes death in twenty-four hours. When adsorption compounds of emetin were introduced into the peritoneal cavities of guinea-pigs, emetin was demonstrable in the urine, as much as twenty-four per cent. being recovered in the urine of a guinea-pig which had received 60 mg. of emetin in the form of the 10 per cent. adsorption compound.

A series of experiments was carried out to demonstrate that soaps and fatty acids may play a rôle in releasing the ipecac alkaloids from their adsorption compound. While large doses of 3.6 per cent. Alcresta ipecac may be administered to cats without causing emesis, on addition of soap or even fatty acid, considerably smaller doses of the adsorbed alkaloid caused vomiting. Adsorbed ipecac alkaloids taken by mouth in moderate doses for three or four days usually produce a laxative effect in man, while larger doses are cathartic. The data accumulated by Bass, Stephens and others indicate the release of the ipecac alkaloids from their adsorption compound in the intestinal tract. One of us (Walters[5]) has personally studied and observed the destructive effect exerted on endamebas in pyorrhea lesions by ipecac alkaloids derived from the 3.6 per cent. adsorption compound administered orally. Space limitations preclude a detailed discussion of the available clinical and pharmacologic data; but it is clear that clinicians who have employed the adsorption compound have generally agreed that a larger dose is required to produce a given effect than is the case when emetin hydrochlorid is employed. The data presented by Fantus,[6] Eggleston and Hatcher,[7] referred to by Sollmann, appear to indicate that lack of emetin symptoms, delay in development of pharmacologic effects, and increased dosage required are all factors which may well be attributable to a delayed release of the alkaloid and the avoidance of peak concentrations.

## EXTRACTION EXPERIMENTS

It has been demonstrated in the preceding experiments that alkaloids adsorbed on Lloyd's reagent in such a manner that they could not be released by extraction with water could nevertheless be extracted, at least to a certain extent, by living tissues. It now remains to be determined whether this extractive capacity is dependent on the use of living protoplasm or whether similar results may be obtained by utilizing substances occurring in protoplasm, particularly lipoids, soaps, etc., or by egg yolk, in which alkaloids might be expected to be more soluble than in distilled water.

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[5] Walters, A. L.; Baker, W. F., and Koch, E. W.: Pharmacological Studies of the Ipecac Alkaloids and some Synthetic Derivatives of Cephaeline, J. Pharmacol. & Exper. Therap. 10; 341 (Nov.) 1917.

[6] Fantus, Bernard: Fullers' Earth: Its Adsorptive Power and Its Antidotal Value for Alkaloids, J. A. M. A. 64: 1838 (May 29) 1915.

[7] Eggleston and Hatcher: J. Pharmacol. & Exper. Therap. 7: 241, 1915.

Soap was found to be an excellent solvent for quinin and other alkaloids. From our data it may be stated that the addition of 1 per cent. soap raises the solubility of quinin in water tenfold, and 16 per cent. soap raises the solubility ninetyfold.

TABLE 4.—ALKALOID EXTRACTION WITH SOAP*

| | Alkaloid Contained in Adsorption Compound | | | | |
|---|---|---|---|---|---|
| Amount Extracted by: | Quinin, 4.8% | Atropin, 2.5% | Strychnin, 5% | 7.5% | Ipecac Alkaloids, 3.6% |
| Water | Nil | Nil | Nil | Nil | Nil |
| 1% sodium bicarbonate PH approximately 8 | Trace | Nil | Nil | Nil | Nil |
| N/10 sodium oleate | 56.6% | 20.7% | 15.3% | ----- | 10.5% |
| N/5 sodium oleate + oleic acid to PH 7.2 to 7.5 | 32.4% | 9.7% | 8.8% | 11.9% | 6.0% |
| | 32.2% | 8.5% | 8.8% | ----- | 7.8% |

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* In each experiment, 2 gm. of the adsorption compound of the alkaloid on Lloyd's reagent was subjected to two extractions with 25 c.c. of solvent, the total amount of alkaloid extracted being recorded in the table as a percentage of the total amount originally present in the adsorption compound.

It has been noted repeatedly in the preceding section that soaps and fatty acids facilitate the release of alkaloids from their adsorption compounds in vivo. Since soap is a good solvent for alkaloids, it might well be expected that it would also facilitate extraction in vitro, and this was found to be the case. A comparison was made of the relative facility with which various alkaloids were extracted from their adsorption compounds on fullers' earth by water, weak alkali of the strength selected by Sollmann, and various soap solutions. Quinin was most readily extracted by means of soap, and atropin, strychnin and emetin less readily; but in all cases the yield of alkaloid was considerable, the ratios observed suggesting some correspondence with those observed in the preceding animal experiments. It is particularly interesting to note that soap, to which oleic acid had been added in order to reduce its alkalinity below that of the 1 per cent. sodium bicarbonate solution used by Sollmann, was nevertheless an excellent solvent, removing 32 per cent. quinin and from 7 to

10 per cent. of the other alkaloids, while the sodium bicarbonate solution extracted only negligible traces.

While it is not within the scope of this paper to discuss at great length the extractive action of soap on these adsorption compounds, it may be stated that when higher concentrations were employed, as much as 90 or 95 per cent. of the alkaloid present could be removed from a 5 per cent. adsorption compound by two or three extractions. Under similar experimental conditions to those reported above:

Egg yolk extracted 10 per cent. ipecac alkaloids and 13 per cent. quinin.

Horse corpuscles extracted 6 per cent. ipecac alkaloids and 9 per cent. quinin.

Intestinal contents extracted more than 1 per cent. of all alkaloids tested.

Macerated intestinal mucosa extracted from 1 to 4 per cent. of all alkaloids tested.

The foregoing results lend support to the theory previously advanced, that soaps, fatty acids, lipoids, etc., present in or near the surface of the cell protoplasm, facilitate a direct extraction of the alkaloid from the Lloyd's reagent.

It is believed that the experimental data presented above afford substantial evidence that the alkaloids atropin, quinin, strychnin and emetin, adsorbed on Lloyd's reagent, are extracted in the intestinal tract and thus rendered therapeutically available.

## SUMMARY

It has been demonstrated that alkaloids, adsorbed on fullers' earth in such a manner that they cannot be extracted by water or an aqueous solution of sodium bicarbonate having the alkalinity of intestinal contents, are released in the intestinal tract when administered orally, and that this release is not dependent on intestinal alkalinity.

The proofs of this are:

Adsorbed atropin administered to cats by mouth produced prolonged dilatation of the pupils of the eye.

Adsorbed strychnin administered by mouth caused convulsions and death in rabbits and rats.

Adsorbed strychnin introduced into the peritoneal cavity of rats caused convulsions and death.

Adsorbed emetin administered orally in large doses caused the death of cats; introduced into the peritoneal cavity it caused the death of rats. Emetin was excreted in the urine when the adsorbed alkaloid was administered orally or introduced into the peritoneal cavity. Adsorbed emetin administered orally caused diarrhoea in men and animals.

Quinin was recovered in considerable amount in the urine after oral administration of the adsorbed alkaloid.

In all of the foregoing cases the dose of a given adsorbed alkaloid required to produce a specific effect was found to be larger than that of the free alkaloid, and it was demonstrated that the rate of absorption was retarded and the action of the alkaloid prolonged, thus avoiding a peak concentration.

While the alkaloids were not extracted from their adsorption compounds by water or a 1 per cent. aqueous sodium bicarbonate solution, they were extracted with considerable facility by slightly alkaline and neutral soaps.

The alkaloids were extracted to a certain extent by egg yolk, blood corpuscles, plasma, intestinal contents and macerated intestinal mucosa.

The foregoing data agree with Sollmann's findings that the ipecac alkaloids are not released from "Alcresta Ipecac" by extraction with a 1 per cent. aqueous sodium bicarbonate solution in vitro, but they do refute the inference drawn by Sollmann that the ipecac alkaloids are not liberated in the intestine when administered orally in the form of this adsorption compound.

The writers desire in conclusion to express their indebtedness to Mr. George B. Walden, Miss E. L. Tevis, Mr. E. H. Stuart and Miss E. Bachman for their cooperation in the experimental portion of this work.

# NEW BENZYL ESTERS POSSESSING AN ANTI-SPASMODIC ACTION.

BY H. A. SHONLE AND P. Q. ROW

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The anti-spasmodic action of the combined opium alkaloids has been shown by Macht[1] to be due to the presence of the benzyl nucleus in the alkaloids of the papaverine group. Simpler compounds containing the benzyl nucleus have the same action. Thus benzyl alcohol, some of its esters, and benzaldehyde have been shown to possess anti-spasmodic properties. Veratryl and piperonyl alcohols, piperonal, vanillin, and veratryl aldehyde, and *o*- and *p*-hydroxy-benzaldehyde possess this action to a higher degree than do benzyl alcohol and benzaldehyde.[2] This inhibitory action present in benzyl alcohol and benzaldehyde is lost in benzoic acid. Apparently the benzyl group acts upon the muscle cell itself and not upon the ends of the nerve fiber in the muscle.

Considerable attention has been directed to the therapeutic value of some of the known esters of benzyl alcohol. As no information was available, however, concerning the benzyl esters of the higher fatty acids, of the amino-sulfonic acids, of the aliphatic and aromatic amino acids, or of the sulfo-benzoic acids, the benzyl esters of a number of these acids were prepared, and their physical properties and their therapeutic activity determined.[3]

Benzyl laurate, myristate, palmitate, stearate, and oleate were first prepared. The benzyl esters of the saturated higher fatty acids are tasteless, orderless liquids or low melting solids. The esters of both the saturated and unsaturated higher fatty acids are insoluble in water but are readily soluble in chloroform, ether, and benzene; and somewhat less soluble in alcohol, olive oil and petroleum oil. The slight but distinct local anesthetic effect noticeable in the lower esters, as well as their irritating action on the mucous membrane was absent in the higher esters, due presumably to their low solubility. Because of the insolubility of these esters the usual physiological tests

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[1] Macht, *J. Pharmacol.*, 9, 287 (1917); 11, 263 (1918).

[2] Unpublished work from the Department of Experimental Medicine.

[3] In preparing these esters the desirability of securing a solid ester free from a disagreeable taste or irritating effect upon the mucous membrane was always considered.

on animals or on excised tissues were not entirely satisfactory as a means of estimating their relative activity on smooth muscle structure. Clinical tests were, therefore, resorted to which demonstrated the anti-spasmodic action of these esters in asthma, dysmenorrhea, high blood pressure, pylorospasm and spastic constipation.

While these esters are very resistant to hydrolysis by steam, they are readily hydrolyzed by the action of lipase. In one case the benzyl ester of commercial stearic acid was hydrolyzed to the same extent as was the olive oil which was used as a control. When the aliphatic acids are replaced by the aromatic acids a very marked reduction of the rate of hydrolysis occurs. Fig. 1 gives the results in graphic form.

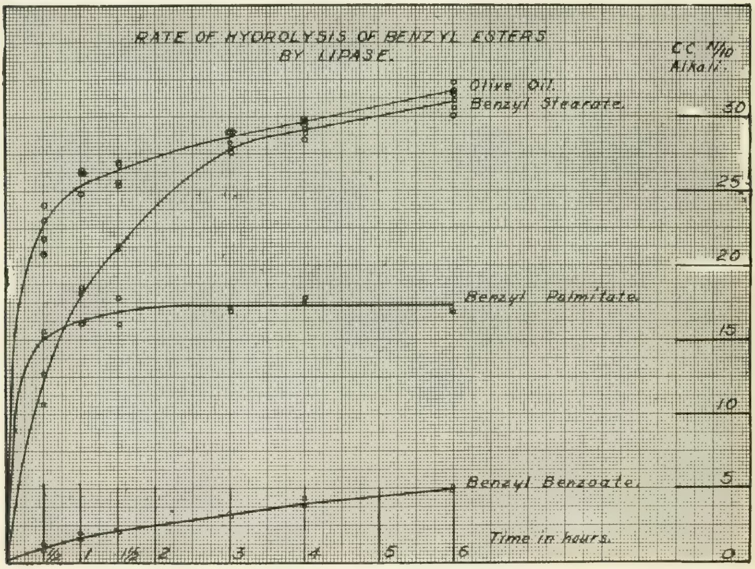

Fig. 1.

Of the esters of the aromatic amino benzoic acids, benzyl *p*- and *m*- amino-benzoates were prepared. Because of the insolubility of their hydrochlorides and because of their irritating effect on mucous membrane, they were not tested for antispasmodic action. Both esters possess local anesthetic properties.

Benzyl lactate was prepared but because of its irritating effect was not tested clinically.

The benzyl esters of the individual fatty acids such as stearic or palmitic have nothing to recommend their therapeutic use over that of the benzyl esters of mixtures of fatty acids derived from naturally occurring sources; consequently various fats and oils were saponified and the benzyl esters of the resulting mixtures of fatty acids were prepared. Since it was found that mixtures of fatty acids containing any considerable amount of unsaturated fatty acids formed colored esters which possess an unpleasant taste, attention was given to the preparation of the benzyl esters of those acid mixtures which were saturated, and especially to the preparation of the benzyl esters of commercial stearic acid. These mixed esters were found to be identical in therapeutic action with those of the individual esters.

## EXPERIMENTAL

The benzyl alcohol used was prepared partly from benzaldehyde by the Cannizzaro reaction and partly by boiling benzyl choride with a solution of sodium carbonate.

## PREPARATION OF THE ACID CHLORIDES AND OF THE BENZYL ESTERS

The chlorides of the fatty acids used in the preparation of the benzyl esters were formed by treating the acids either with phosphorus pentachloride or thionyl chloride. About 10% excess of the theoretical amount of phosphorus pentachloride was added to the acid and the reaction allowed to proceed at room temperature. When the reaction was completed, the phosphorus trichloride formed was removed by vacuum distillation and the resulting acid chloride purified by distilling at a pressure of 10 mm. to 20 mm. In no case was it possible to secure more than 50% of the theoretical yield by this method, while the usual yield was about 30%.

An unusuccessful attempt was made to replace phosphorus pentachloride with phosphorus trichloride. Although a reaction occurred, all attempts to separate any acid chloride by vacuum distillation failed.

Thionyl chloride was found to be the best chlorinating agent. Five times the theoretical amount of thionyl chloride was added to the acid and the reaction allowed to proceed at room temperature. Upon its completion the reaction flask was placed on the water-bath and the excess of thionyl chloride was distilled off. The acid chloride secured in this manner was sufficiently pure to be used without further treatment.

The esters were prepared by allowing benzyl alcohol to react with an ethereal solution of the acid chloride prepared by one of the 2 above methods. They were subsequently purified either by crystallization from alcohol or by vacuum distillation.

*Benzyl laurate.*—The lauric acid used was prepared from the fatty acids of coconut oil and had a melting point of 43.3°.[1] Benzyl laurate boils at 209-211° (12 mm.), melts at 8.5°, has d25-25 0.9457, and $n^{24}$ 1.4812.

*Benzyl myristate.*—The myristic acid was prepared by saponifying the colored fat secured by exhaustively extracting ground nutmegs with ether and alcohol.[1] After recrystallizing 3 times from alcohol, the acid melted at 54-55° and had a molecular weight of 229. Four pounds of nutmegs gave 132 g. of the pure acid. Benzyl myristate boils at 229-231° (11 mm.), melts at 20.5°; has d25-25 0.9321; and $n^{24}$ 1.4803.

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[1] Secured from the Department of Chemistry, University of Illinois.

*Benzyl palmitate.*—The palmitic acid was prepared by saponifying bayberry wax and recrystallizing the acid 4 or 5 times from alcohol.[1] The yield was about half the initial weight of the wax. It has a melting point of 62-63° and a molecular weight of 259. Benzyl palmitate melts at 36.0°; has d38-25 0.9136; and $n^{50}$ 1.4689.

*Benzyl stearate.*—The stearic acid was prepared by saponifying cocoa butter and recrystallizing the acids secured from alcohol until a constant melting point was secured.[2] Ninety-five g. of stearic acid with a melting point of 68-69° and a molecular weight of 283 was secured from one kg. of cocoa butter. Benzyl stearate melts at 45.8°; has d50-25 0.9075; and $n^{50}$ 1.4663.

*Benzyl oleate.*—The oleic acid was prepared from tallow by the usual lead salt method. The acid secured had an iodine value of 91.5 and a molecular weight of 284. Benzyl oleate boils at 237° (7 mm.), and is liquid at 0°. It has d25-25 0.9330, and $n^{25}$ 1.4875.

*Benzyl lactate.*—This ester was prepared by heating benzyl alcohol with lactic acid which had previously been freed from water by heating to 180°. Dry hydrogen chloride was used as a catalyzer. It was found necessary to keep the temperature between 220° and 230° for 10 to 12 hours in order to secure esterification. Upon fractionation, benzyl lactate was secured, in the fraction boiling at 128-130° (5-6 mm.). The ester has $n^{25}$ 1.5252.

*Benzyl amino Benzoates.*—The benzyl ester of *p*-amino-benzoic acid was prepared by allowing theoretical amounts of *p*-nitro-benzoyl chloride to react with benzyl alcohol in dry ether. The theoretical yield of the nitro-ester melting at 84.7° was secured. The amino ester was secured by reducing the latter either by tin in alcoholic hydrochlorid acid or by powdered iron and hydrochloric acid. The hydrochloride of this ester is a white crystalline solid melting at 184° with the formation of a slight amount of gas. On analysis 13.41% of chlorine was found, the calculated amount being 13.47%. The free base is a yellow viscous liquid having no definite melting point and becoming wax-like on standing. Neither the ester, the ester hydrochloride, nor lactate was appreciably soluble in water.

Benzyl-*m*-amino-benzoate was prepared in the same manner. The benzyl-*m*-nitro-benzoate is a light yellow liquid which boils at 308° (760 mm.). The amino ester is a dark liquid forming a crystalline hydrochloride which has no definite melting point.

*Other Benzyl Esters.*—The benzyl ester of *o*-sulfo-benzoic acid could not be prepared either from the acid choloride or from the alcohol and the acid. In both cases a syrup-like material was secured which could not be resolved into a definite substance.

Glycocoll, heated at 120° to 140° with an excess of benzyl alcohol in the presence of a stream of dry hydrogen chloride for 8 hours, was not esterified.

*The Rate of Hydrolysis of Benzyl Esters by Lipase.*—A 5% acacia emulsion was made of the ester and the aqueous extract of fresh pancreas in such a manner that each 20 g. of the emulsion contained 2.25 g. of the ester, the extract of one g. of pancreas, and 0.15 g. of sodium tauroglycocholate. This emulsion was incubated in 20-g. duplicate samples for the desired periods of time. During the incubation, the samples were frequently agitated since the emulsion showed a slight tendency to break up. At the end of the incubation period, the samples were removed and immersed in boiling water for 10 minutes. They were then cooled and 60 cc. of a neutral mixture of 5 parts of alcohol and one part of ether was added. They were titrated with 0.1 *N* sodium hydroxide, using phenolphthalein as an indicator. The end-point was taken as that point at which the pink color persisted for 30 seconds.

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[1] Prepared according to directions kindly furnished by Dr. G. D. Beal, University of Illinois.

[2] Hehner and Mitchell, Journal of the American Chemical Society, 19, 32 (1897).

A control was run with olive oil in place of the ester and a blank was made up in the same manner but containing neither the oil nor the ester. In this way correction was made for the hydrolysis of the fat contained in the pancreatic extract. The results are reported in terms of 0.1 *N* alkali required.

TABLE 1.—RATE OF HYDROLYSIS OF BENZYL ESTERS BY LIPASE.

(Results in cc. of 0.1 *N* alkali).

| Time: hours. | ½ | 1 | 1½ | 3 | 4 | 6 |
|---|---|---|---|---|---|---|
| Olive oil | 23.0 | 26.5 | 26.7 | 28.9 | 29.5 | 31.6 |
| 5-5-20 | 24.0 | 26.4 | 26.8 | 28.8 | 29.8 | 31.7 |
| Olive oil | 20.6 | 24.9 | 26.2 | 28.7 | 29.4 | 32.3 |
| 5-6-20 | 21.8 | 26.4 | 25.5 | 27.6 | 28.4 | 27.6 |
| Benzyl stearate | 10.6 | 18.2 | 21.0 | 28.2 | 29.2 | 31.0 |
| 5-5-20 | 12.9 | 18.5 | -- | 27.7 | 29.0 | 30.6 |
| Benzyl palmitate | 15.5 | 16.1 | 16.0 | 16.9 | 17.5 | 16.8 |
| 5-6-20 | 15.0 | 16.0 | 17.8 | 17.2 | 17.7 | 16.8 |
| Benzyl benzoate | 1.2 | 1.9 | 2.1 | 3.1 | 3.9 | 4.8 |
| 5-6-20 | 0.9 | 2.0 | 2.1 | 3.1 | 4.1 | 4.8 |

## REACTION OF BENZYL CHLORIDE WITH THE SODIUM SALTS OF THE HIGHER FATTY ACIDS

The usual laboratory methods of esterification are not feasible for the production of large amounts of the benzyl esters of the higher fatty acids. It was found, however, that such benzyl esters could be readily prepared by allowing benzyl chloride to react with the anhydrous alkali salt of the fatty acid dissolved in an excess of that acid.

The acid was first heated to 150-170° and half neutralized with anhydrous or mono-hydrated sodium carbonate. At this temperature the soap formed was completely dissolved. Benzyl chloride was then added in an amount sufficient to react completely with the sodium salt and the temperature kept at 170° to 180° for several hours when the reaction was completed. Half of the remaining acid was then neutralized and an amount of benzyl chloride sufficient to react with it added. By repeating this process the acid can be converted into the benzyl ester without the addition of any solvent or catalyzer. The temperature should not be allowed to go above 200° and the mixture must be stirred to prevent foaming and charring.

When the esterification is completed the sodium chloride is removed by washing with water, the ester is crystallized from alcohol, and any trace of benzyl chloride left is removed by steam distillation.

Commercial stearic acid having a melting point of 55-56° and an iodine value of 2 to 3 was esterified by this method. The resulting benzyl stearate which melts at 30-34° can be resolved into fractions melting from 12° to 39° by fractional crystallization from alcohol.[1]

## SUMMARY

1. Methoxy and hydroxy derivatives of benzyl alcohol

and benzaldehyde possess marked anti-spasmodic action.

2. The benzyl esters of the higher fatty acids are tasteless, odorless liquids or low-melting solids possessing anti-spasmodic action. They are more readily hydrolyzed by lipase than are the benzyl esters of aromatic acids.

3. Benzyl chloride readily reacts with the alkali salts of the higher fatty acids when they are dissolved in the hot acid, forming the benzyl ester of these acids.

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[1] In the course of this investigation the following new compounds were prepared and identified, benzyl laurate, benzyl myristate, benzyl palmitate, benzyl stearate, benzyl oleate, benzyl lactate, benzyl-*p*-amino-benzoate, benzyl-*m*-nitro-benzoate, and benzyl-*m*-amino-benzoate.

# ATROPINE SULFATE FROM DATURA STRAMONIUM[1]

BY H. W. RHODEHAMEL AND E. H. STUART

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*Datura stramonium,* commonly known as Jimson weed, grows in almost all parts of the United States and Europe.

Atropine was first isolated from this plant in 1833 by Geiger and Hesse,[2] but hyoscyamine is the chief alkaloidal constituent. The latter is isomeric with atropine, and is converted into it during the process of extraction and purification. The percentage of total alkaloids varies from 0.15 to 0.6 per cent in the dried plant, the variation depending mainly on the age and vigor of growth of the plant. The moisture content of the whole plant is from 75 to 85 per cent. Assays made of the plant in flower showed the distribution of alkaloid to be as follows:

| | Per cent. |
|---|---|
| Stems below the first fork | 0.054 |
| Stems above the first fork | 0.069 |
| Seed pods without seeds | 0.054 |
| Seeds | 0.45 |
| Leaves | 0.414 |

The comparatively small amount of alkaloids occurring in *Datura stramonium* made their commercial extraction impracticable until the discovery in 1913 by J. U. Lloyd that under certain conditions fuller's earth would adsorb alkaloids from dilute solutions of their salts. The entire green plant was ground, percolated with acidulated water, and to the percolate Lloyd's reagent was added. After drying, the Lloyd's reagent contained by assay from 2 to 2.5 per cent alkaloidal material. In this manner a considerable concentration of the alkaloids was effected.

## COMMERCIAL EXTRACTION OF THE ALKALOID

The stramonium plant was harvested from about the middle of July to the first hard frost, which usually occured during October. The entire plant, except the roots, was ground in the green state, packed in large wooden tanks, and percolated with water containing 0.2 per cent sulfuric acid and 0.5 per cent formaldehyde. Maceration was allowed to continue 3 days, and the rate of percolation controlled so that about 300 gal. each day were obtained from a 4000-gal. tank.

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[1] Presented before the Division of Medicinal Products Chemistry at the 60th Meeting of the American Chemical Society, Chicago, Ill., September 6 to 10, 1920 and reprinted from the Journal of Industrial and Engineering Chemistry, March, 1921, Vol. 13, page 218.

[2] *Ann.,* 5, 43; 6, 44; 7, 269.

Fifteen hundred gallons of the dilute atropine sulfate solution were treated at one time with Lloyd's reagent. The minimum amount of the latter necessary for maximum adsorption was controlled by assay of the percolate prior to its addition. To insure adequate agitation, air was blown into the mixture for about 20 min. It required about 12 to 14 hrs. for the Lloyd's reagent to settle, after which the exhausted percolate was decanted, and the precipitate drained and thoroughly dried at a temperature of about 50° C.

| Portion | Percolate Collected Gallons | Alkaloidal Material per 100 Cc. Grams | Alkaloidal Material in Percolate Pounds | Percentage of Total Alkaloidal Material Removed | Lloyd's Reagent Added Pounds |
|---|---|---|---|---|---|
| | | *Tank 1*[1] | | | |
| 1 | 1266 | 0.0123 | 1.30 | 10.75 | 60 |
| 2 | 1500 | 0.0107 | 1.34 | 11.08 | 60 |
| 3 | 1500 | 0.0075 | 0.94 | 7.77 | 45 |
| 4 | 1500 | 0.0042 | 0.53 | 4.38 | 20 |
| Total | | | 4.11 | 33.98 | 185 |
| Assay of extracted drug gave 0.07 per cent. alkaloid. | | | | | |
| | | *Tank 2*[2] | | | |
| 1 | 1600 | 0.03676 | 4.91 | 59.28 | 230 |
| 2 | 900 | 0.0319 | 2.40 | 28.98 | 110 |
| 3 | 1500 | 0.0028 | 0.35 | 4.23 | 16 |
| 4 | 950 | 0.0006 | 0.05 | 0.57 | 3 |
| Total | | | 7.71 | 93.06 | 359 |
| Assay of extracted drug gave 0.024 per cent. alkaloid. | | | | | |
| | | *Tank 3*[3] | | | |
| 1 | 1500 | 0.0251 | 3.14 | 25.99 | 150 |
| 2 | 1500 | 0.0143 | 1.79 | 14.81 | 90 |
| 3 | 1500 | 0.0071 | 0.89 | 7.35 | 45 |
| 4 | 1500 | 0.0018 | 0.23 | 1.36 | 12 |
| Total | | | 6.05 | 50.01 | 297 |
| Assay of extracted drug gave 0.054 per cent. alkaloid. | | | | | |

| No. of Tanks | Packed in | Average Percentage of Alkaloid Present |
|---|---|---|
| 10 | July | 40.69 |
| 11 | August | 69.03 |
| 8 | September | 58.89 |
| 7 | October | 39.02 |

Average for the year 51.9 per cent.

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[1] Packed July 15 with 26,000 lbs. of green stramonium. Moisture 85 per cent. Assay of dried sample gave 0.31 per cent. alkaloidal material. From the assay the tank contained 12.09 lbs. alkaloidal material.

[2] Packed August 23 with 15,500 lbs. of green stramonium. Moisture 85 per cent. Assay of dried sample gave 0.356 per cent. alkaloidal material. From the assay the tank contained 8.28 lbs. of alkaloidal material.

[3] Packed October 4 with 26,000 lbs. of green stramonium. Moisture 85 per cent. Assay of dried sample gave 0.31 per cent. alkaloidal material. From the assay the tank contained 12.09 lbs. alkaloidal material.

Results obtained on a great number of tanks would be of no special interest. The percolation records of three tanks have therefore been selected to show, first, the poor adsorption obtained when using young drug harvested in July; second, the best adsorption from August drug; and third, the decrease in adsorption in October drug.

### DETERMINATION OF ADSORBED ALKALOID

The adsorbed alkaloidal material was determined as follows: Two grams of the Lloyd's reagent containing the alkaloid were placed in a glass-stoppered bottle, and 40 cc. ether and 3 to 5 cc. 16 per cent. ammonia water were added. The contents were thoroughly agitated, and after settling the ether was decanted. The ether extraction was repeated six times, and the extracts combined, filtered, and evaporated to dryness. Ten cc. 0.05 *N* sulfuric acid were added, and the excess acid titrated with 0.02 *N* sodium hydroxide solution, using cochineal as the indicator.

Determination of the alkaloid remaining in the Lloyd's reagent after extraction was made in the same way, except that a larger sample was used. The reagent after extraction always contained about 0.2 per cent alkaloidal material which could not be economically removed.

| Portion | Percolate Gallons | Alkaloidal Material per 100 Cc. Grams | Alkaloidal Material in Percolate Pounds | Percentage of Total Alkaloidal Material Removed | Lloyd's Reagent Added Pounds |
|---|---|---|---|---|---|
| | | *Barrel 1*[1] | | | |
| 1 | 36 | 0.01721 | 0.0517 | 41.87 | 2.6 |
| 2 | 36 | 0.0085 | 0.0255 | 20.68 | 1.3 |
| 3 | 36 | 0.0046 | 0.0138 | 11.18 | 0.7 |
| Total | | | 0.0910 | 73.73 | 4.6 |

Assay of exhausted drug gave 0.02 per cent. alkaloid. 4.6 lbs. Lloyd's reagent recovered. Assay 1.99 per cent. 74.09 per cent. of the alkaloidal material was removed by the Lloyd's reagent.

| Portion | Percolate Gallons | Alkaloidal Material per 100 Cc. Grams | Alkaloidal Material in Percolate Pounds | Percentage of Total Alkaloidal Material Removed | Lloyd's Reagent Added Pounds |
|---|---|---|---|---|---|
| | | *Barrel 2*[2] | | | |
| 1 | 36 | 0.01336 | 0.0401 | 32.50 | 2.0 |
| 2 | 36 | 0.0087 | 0.0261 | 21.16 | 1.3 |
| 3 | 36 | 0.0060 | 0.0180 | 14.60 | 0.9 |
| Total | | | 0.0842 | 68.26 | 4.2 |

Assay of exhausted drug gave 0.048 per cent. alkaloid. 4.2 lbs. Lloyd's reagent recovered. Assay 1.97 per cent. 67.00 per cent. of the alkaloidal material was removed by the Lloyd's reagent.

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[1] The menstruum was water containing 25 g. of bleaching powder and 10 cc. of sulfuric acid per gallon.

[2] The menstruum was water containing 8 cc. of cresol and 7 cc. of sulfuric acid per gallon.

*Barrel 3*[3]

| | | | | | |
|---|---|---|---|---|---|
| 1 | 36 | 0.0148 | 0.0445 | 36.05 | 2.25 |
| 2 | 36 | 0.0077 | 0.0231 | 18.73 | 1.15 |
| 3 | 36 | 0.0028 | 0.0084 | 6.81 | 0.4 |
| Total | | | 0.0760 | 61.59 | 3.8 |

Assay of exhausted drug gave 0.036 per cent. alkaloid. 3.9 lbs. Lloyd's reagent recovered. Assay of 2.05 per cent. 64.74 per cent. of the alkaloidal material was removed by the Lloyd's reagent.

*Barrel 4*[4]

| | | | | | |
|---|---|---|---|---|---|
| 1 | 36 | 0.0127 | 0.0381 | 30.89 | 1.9 |
| 2 | 36 | 0.0048 | 0.0144 | 11.68 | 0.72 |
| 3 | 36 | 0.0034 | 0.0102 | 8.27 | 0.80 |
| Total | | | 0.0672 | 50.84 | 3.12 |

Assay of exhausted drug gave 0.032 per cent. alkaloid. 3.25 lbs. Lloyd's reagent recovered. Assay 1.97 per cent. 51.84 per cent. of the alkaloidal material was removed by the Lloyd's reagent.

Differences in age of the plant and condition in growth caused some variations in the extraction of the alkaloidal material. The plants harvested in July were about 2 ft. high and just beginning to flower. The alkaloidal content of the young plants was 0.1 per cent higher than those gathered in August, and considering this, the July plants should have yielded more atropine per pound of drug than did the August plant. This, however, was not the case. Taking into consideration the amount of alkaloid obtained and the amount actually present in the plant, the average yield for July was 28.5 per cent lower than that obtained in August. Since the drug after extraction showed only negligible amounts of alkaloid, it was assumed that a considerable proportion of alkaloid was decomposed during percolation. Examination of the exhausted drug showed no evidence of decomposition resulting from bacteria or molds. Possibly the loss was due to an enzymic action, although this point was not investigated.

A considerable number of small experiments were made with the attempt to increase the yield of alkaloid. A menstruum containing acetic, hydrochloric or sulfuric acid in various concentrations gave no better results and showed no marked difference. Somewhat better results were obtained, however, when other preservatives were substituted for the formaldehyde. Results on four of these experiments are given. With the usual formaldehyde preservative 50.84

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[3] The menstruum was water containing 30 cc. of 40 per cent. solution of sodium bisulfite and 10 cc. sulfuric acid per gallon.

[4] The menstruum was water containing 18 cc. of formaldehyde solution and 7 cc. sulfuric acid per gallon.

per cent of the total alkaloidal material was extracted; with sodium bisulfite as the preservative 61.59 per cent; with cresol 68.26 per cent; with bleaching powder 73.73 per cent.

A number of barrels were packed September 20 with green stramonium, each containing 260 lbs. Moisture 80.8 per cent. Assay of dried sample gave 0.24 per cent. alkaloidal material. Accordingly, the drug in each barrel contained 0.1235 lb. of alkaloidal material. Percolated at the rate of 12 gal. per day.

### EXTRACTION OF THE ALKALOID FROM LLOYD'S REAGENT

In a general way the alkaloidal material was obtained from the Lloyd's reagent by first adding water, then making the mixture alkaline, and subsequently extracting with an organic solvent. It was very essential to make the mixture alkaline with a base that would not decompose the alkaloid. Ammonia and lime water were found to be the best for this purpose. Three methods were used for the extraction. First, ammonia water and ether; second, lime water and ether; and third, lime and alcohol. The extractions were made in a revolving drum.

METHOD 1—To 300 lbs. of Lloyd's reagent containing the alkaloid, 35 gal. of 16 per cent ammonia were added. This gave a rather thick paste, and on revolving the drum the material was carried part way up the sides, then fell back, and in this way was thoroughly mixed with the ether. Each extraction was continued for 45 min. The results were as follows:

| Extraction | Ether Used Gal. | Alkaloidal Material Removed Oz. |
|---|---|---|
| 1 | 200 | 13.5 |
| 2 | 130 | 7.1 |
| 3 | 120 | 9.4 |
| 4 | 120 | 8.2 |
| 5 | 120 | 5.3 |
| 6 | 150 | 8.0 |
| 7 | 140 | 6.1 |
| 8 | 120 | 4.9 |
| 9 | 120 | 4.1 |
| 10 | 120 | 3.8 |

An assay of the extracted Lloyd's reagent gave 0.45 per cent alkaloid, showing that 22.5 per cent of the alkaloidal material still remained in it.

METHOD 2—Same as Method 1, except that in place of 16 per cent. $NH_3$, 40 lbs. of CaO in 35 gal. of water were added.

An assay of the extracted Lloyd's reagent gave 0.29 per cent alkaloid, showing that 13 per cent of the alkaloidal material still remained in it.

| Extraction | Ether Used Gal. | Alkaloidal Material Removed Oz. |
|---|---|---|
| 1 | 160 | 14.22 |
| 2 | 140 | 9.41 |
| 3 | 145 | 8.62 |
| 4 | 160 | 7.66 |
| 5 | 150 | 5.80 |
| 6 | 150 | 5.80 |
| 7 | 150 | 5.44 |
| 8 | 150 | 2.89 |
| 9 | 150 | 5.33 |
| 10 | 150 | 4.41 |

METHOD 3—To 300 lbs. Lloyd's reagent (assay 2.36 per cent) 40 lbs CaO were added and the mixture extracted for about 3 hrs. with the following volumes of 80 per cent alcohol:

| 80 Per Cent. Alcohol Used Gal. | Alkaloidal Material Removed Oz. |
|---|---|
| 175 | 45.44 |
| 150 | 17.61 |
| 150 | 13.68 |
| 150 | 8.92 |
| 150 | 7.42 |

An assay of the extracted Lloyd's reagent gave 0.18 per cent alkaloid, showing that 7.6 per cent of the alkaloidal material still remained in it.

The following is of interest in connection with the alcohol extraction of the alkaloidal material from Lloyd's reagent.

A mixture of 10 g. of Lloyd's reagent with 1.3 g. of slaked lime was extracted for 14 hrs. with 50 cc. of alcohol of the following strengths:

| Strength of Alcohol Per cent. | Alkaloidal Material Removed Per cent. |
|---|---|
| 100 | 0.00 |
| 90 | 25.42 |
| 80 | 41.25 |
| 70 | 41.25 |
| 60 | 33.82 |
| 50 | 23.50 |

The volume of the 80 per cent. alcohol was varied as follows:

| Alcohol Used Cc. | Alkaloidal Material Removed Per cent. |
|---|---|
| 20 | 26.8 |
| 30 | 32.6 |
| 40 | 35.0 |
| 50 | 41.25 |
| 80 | 43.6 |
| 100 | 47.0 |

## PURIFICATION OF THE CRUDE ATROPINE

The alkaloidal material was extracted from the Lloyd's reagent with 95 per cent alcohol, using lime to obtain the proper alkalinity. The extractions were acidulated with acetic acid and the solution concentrated first to 12 per cent, and then under diminished pressure to 2 per cent of its original volume. This procedure was sufficient to convert all the hyoscyamine into atropine. After neutralization with ammonia, the solution was allowed to stand over night and filtered. A test portion of the filtrate was shaken with ether. If an emulsion resulted, the solution was diluted about one-fourth and returned to the vacuum still. Distilling the neutral liquid, and again filtering, usually prevented the troublesome emulsion with ether. Ammonia was added until the solution was alkaline and the atropine alkaloid extracted with ether. After evaporation of the ether, the alkaloid was carefully dried at about 35° C.

The dried alkaloid was dissolved in ethyl alcohol in the proportion of one ounce of alkaloid to two fluid ounces of solvent, and the solution almost neutralized with sulfuric acid, using cochineal as indicator. After filtering it was evaporated on the water bath to a thin sirup, and to this sirup, while still warm, acetone was added almost to the point of precipitation of the atropine sulfate.

On cooling, the atropine sulfate crystallized. If not sufficiently pure the crystals were dissolved in alcohol and recrystallized as outlined above.

The acetone was evaporated from the mother liquor, and the alcoholic solution of atropine sulfate poured into a large volume of water. From this the alkaloid was extracted with ether, and if not of sufficient purity the process already outlined was repeated.

## CORRECTION

In Series 1, Number 10, July 30, 1920, the article entitled "Contribution to the Pharmacology of Cotton Root Bark", Chas. R. Eckler, M. S., was printed without giving credit to the American Journal of Pharmacy where it was originally published in Volume 92, Page 285.

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